Rationale and Design of the Kronos Early Estrogen Prevention Study (KEEPS) and the KEEPS Cognitive and Affective Sub Study (KEEPS Cog).

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Introduction

Prior to the publication of the Women’s Health Initiative (WHI) and the WHI Memory Study (WHIMS) findings [1–4], hormone therapy (HT) was thought to significantly reduce a woman’s risk for cardiovascular disease, osteoporosis, sexual dysfunction, certain cancers and dementia. This belief was based on a large body of basic science and epidemiological evidence indicating that HT elicited salutary cardiovascular effects and could potentially reduce the risk of Alzheimer’s disease (AD), all while alleviating menopausal symptoms (Hogervorst et al). In 2002, the primary results from the WHI showed no cardiovascular benefit of HT, and the WHIMS indicated an increased risk of dementia. Consequently, findings from observational studies conducted prior to the WHI were attributed to the ‘healthy-user’ bias [5[Matthews, 1996 #5848]. Still, support for the potential beneficial effects of HT on cardiovascular outcomes and cognition continues to mount from observational (including WHIMS data [6]), clinical and prospective cohort studies [7–11]. This manuscript describes the issues raised by the WHI and WHIMS and presents the most recent results and ongoing HT-related research studies designed to address these issues, including the Kronos Early Estrogen Prevention Study (KEEPS) and the KEEPS Cognitive and Affective sub study (KEEPS Cog).

Issues Raised by the WHI and the WHIMS

In 2002, the conjugated equine estrogen + medroxyprogesterone acetate (CEE+MPA) portion of the WHI trial was halted because of a trend toward increased breast cancer, although this relationship was not statistically significant. Soon after, the CEE alone arm of the trial was halted because of a perceived lack of benefit of HT. While terminated early, the WHI was the largest clinical trial ever conducted and served to resolve questions surrounding certain forms of HT administration (i.e. CEE) in older women, while raising important methodical issues driving current HT-related research.
The Critical Period Hypothesis

Arguably the most important issue raised by the WHI was the 'critical period' hypothesis. Before the WHI trials, most data citing cardiovascular benefits of HT came from studies with recently menopausal women, who are most often prescribed HT to combat menopausal symptoms. In contrast, the average age of participants enrolled in the WHI trials was 63 years at HT administration onset, which is 12 years after the average age of menopause in the United States [12, 13]. Because atherosclerosis progresses gradually before declaring itself with a clinical event [60, 64], a significant number of women in the WHI likely had subclinical atherosclerosis at randomization [14]. The presence of subclinical disease might have accounted for the tendency for cardiovascular events to be increased early in the WHI, just as in the cardiovascular prevention study with HT, the Heart and Estrogen/Progestin Replacement Study (HERS), which began concurrently with the WHI. The HERS showed that in women with preexisting disease, there was an increase in cardiovascular events in the first few years of the trial. Although continued use of HT was associated with a reversal of this trend, the major conclusion from that trial was that women with preexisting coronary heart disease were not candidates for HT [14][47].

Hormone Therapy Formulations - Estradiol vs. Estrone

Another major methodological issue raised by the WHI and the WHIMS was the form of HT employed. Oral CEE is the most widely used estrogen for HT in the United States. CEE is a formulation comprised of estrone sulfate and at least ten other hormones, some of which are non-human [4]. Estradiol is comprised of 17β-estradiol, the most potent and natural human form of estrogen in premenopausal women. The estrogen receptor (ER) binding potency of estrone is approximately \( \frac{2}{3} \) the affinity of estradiol to the ER\( \alpha \), and about \( \frac{1}{3} \) the potency of estradiol for ER\( \beta \) [8]. Additionally, estrone and estradiol likely have differential potencies for membrane mediated actions [9]. Thus, estradiol may be more effective than estrone formulations of HT.

If the purpose of HT is to control menopausal symptoms and prevent bone loss, the various forms of HT may be interchangeable [15]. However, if the goal of HT is to achieve a hormone state closer to that observed prior to menopause, in order to maintain cardiovascular and neurobiological systems, administration of an estradiol HT would be preferable to CEE formulations. As discussed, the predominant form of estrogen in premenopausal women is estradiol, the levels of which decline more than estrone levels after menopause. However, in contrast to basic science research, many clinical studies evaluating cognitive efficacy of hormone therapies in healthy older women have utilized CEE. To address this issue, the KEEPS and KEEPS Cog trials examined the extent to which oral CEE and 17β estradiol influenced cardiovascular risk factors and cognition, in addition to addressing the 'critical period' hypothesis.

Route of Hormone Therapy Administration

The CEE used in the WHI and WHIMS was an oral HT formulation [16]. Oral estrogens are subject to extensive hepatic metabolism and result in an estrone to estradiol ratio of approximately 5:1 to 7:1 [10]. Additionally, oral estrogen administration increases the risk of venous thromboembolic (VTE) complications by inducing pro-coagulant proteins during first-pass hepatic metabolism. Some researchers have proposed that opposed CEE-induced cerebrovascular changes might underlie increased risk for dementia described by WHIMS data [11]. In contrast to oral formulations, transdermal estrogen administration bypasses hepatic metabolism and results in a steady-state concentration of estradiol with an estrone : estradiol ratio of 1:1, approximating that seen prior to menopause. Another advantage of transdermal estradiol is that, unlike oral preparations, it does not increase binding
glycoproteins, such as sex hormone binding globulin (SHBG), which results in higher plasma concentrations of free estradiol. Thus, date suggests that not only is HT formulation influential, but the route of administration is also important. KEEPS will provide the first direct comparison of effects of long-term use of oral versus transdermal HT on atherosclerosis in women. Moreover, KEEPS will evaluate mode of treatment delivery similar to what has been used in the majority of basic science studies; namely transdermal delivery for estradiol HT and oral delivery for CEE.

Cyclical Micronized Progesterone vs. Continuous Administration of Medroxyprogesterone Acetate (MPA)

Women who have a uterus are prescribed a progestin in addition to an estrogen because unopposed estrogens markedly increase the risk of endometrial cancer. Some synthetic progestins and their metabolites have been shown to have deleterious cognitive effects [17], either by exerting sedative [18, 19] and adverse mood effects [19], or possibly by accelerating cognitive decline [20]. Evidence from epidemiological studies shows that synthetic progestins, including medroxyprogesterone acetate (MPA), can lead to decreased performance on measures of cognition [17], and a greater rate of cognitive decline [20]. Additionally, there is emerging evidence from basic science research that MPA attenuates the neuroprotective potential of estrogen and fails to protect against glutamate toxicity [21]. Conversely, natural forms of endogenous progesterone may exert beneficial neuropathological and cognitive effects.

Prometrium® is an oral form of progesterone which is bio-identical to the progesterone produced by the ovaries. In contrast to synthetic progestins, Prometrium® has not been shown to be associated with the adverse effects related to heart disease, lipid metabolism or thrombo-embolic diseases, and is available in a micronized formulation to increase bioavailability [22, 23]. While it is unknown if an exogenous gestational agent will act as the endogenous hormone, cyclic administration of a form simulating the endogenous human molecule is theoretically preferred because it more closely approximate the premenopausal hormone milieu compared to chronic dosing with MPA. Because undergoing a hysterectomy was an exclusion for participation the KEEPS trial, all participants were administered combined HT (or placebo). To avoid MPA-related complications discussed above, and to test the efficacy of cyclical administration of bio-identical progesterone, all women in the KEEPS were administered natural, micronized progesterone.

The Kronos Early Estrogen Prevention Study (KEEPS)

To address the above mentioned issues raised by the WHI and WHIMS, investigators conducted a nine-site, randomized, double blind placebo-controlled clinical trial, called the Kronos Early Estrogen Prevention Study (KEEPS; NCT000154180). Our hypothesis was that HT initiated early after menopause (i.e., prior to the appearance of advanced atherosclerotic lesions) would prevent progression of atherosclerosis. Women were randomized if they were aged 42 to 58 years, at least 6 months but no more than 36 months postmenopausal and in good general health with a normal mammogram (See Harman et al. for a detailed study description [24]). Also, only with plasma follicle-stimulating hormone (FSH) level ≥35 ng/mL and/or E2 levels <40 pg/mL were eligible. Women were excluded if they self-reported prior or current cardiovascular disease, including myocardial infarction, angina, congestive heart failure, thromboembolic disease. Exclusion criteria also included smoking more than 10 cigarettes per day, a BMI > 35 mm2/kg, dyslipidemia (LDL cholesterol > 190 mg/dL), hypertriglyceridemia (triglycerides > 400 mg/dL), and uncontrolled hypertension (systolic blood pressure > 150 mm Hg and/or diastolic blood pressure > 95 mm Hg) or glucose > 126 mg/dL. The purpose of the stringent inclusion
guidelines was to ensure that women enrolled in KEEPS and KEEPS Cog did not have existing preclinical vascular disease, which as discussed above, likely contributed to the increased incidence of vascular disease in the WHI, and later development of dementia in the WHIMS.

The primary objective of the KEEPS was to evaluate the differential efficacy of oral CEE (Premarin® 0.45 mg/day) and transdermal 17β estradiol (Climara® 50 µg/day) versus placebo on progression of atherosclerosis as defined by carotid intima–media thickness (CIMT) [25] and coronary arterial calcification (CAC) [26] in women within three years of their last menstrual period. The trial began in July, 2005 with complete enrollment of 728 participants in June 2008. The last study visit took place in March 2012 and manuscript preparation is currently underway. Women meeting inclusion criteria were randomized to daily placebo, oral CEE, or transdermal 17β estradiol with placebo or micronized oral progesterone (Prometrium® 200mg) for the first 12 days of each month. Secondary objectives included a systematic characterization of the effects of HT on measures of inflammation and blood hypercoagulability.

All laboratory and cognitive testing took place at one of nine clinical testing sites including: Brigham and Women’s Hospital, Boston, MA, Columbia University College of Physicians and Surgeons, New York, NY, Mayo Clinic, Rochester, MN, Montefiore Medical Center, Bronx, NY, Kronos Longevity Research Institute, Phoenix, AZ, University of California at San Francisco, San Francisco, CA, University of Utah School of Medicine, Salt Lake City, UT, University of Washington School of Medicine; Tacoma, WA, or Yale University School of Medicine, New Haven, CT. All participants underwent an extensive medical and medication history, ultrasound testing, physical exam, laboratory blood tests, and neuroimaging (at the Mayo clinical center only). A description of testing has been described in a previous publication {Harman, 2005 #2867}.

The KEEPS-Cognitive and Affective Sub Study (KEEPS-Cog)

An ancillary study of KEEPS, funded by the National Institutes of Health’s National Institute on Aging (NIH-NIA), called the KEEPS Cognitive and Affective study (KEEPS Cog) evaluated the differential efficacy of transdermal estradiol and oral CEE on measures of cognitive function and mood in women enrolled in the parent KEEPS study. The collection of baseline cognitive data from over 700 women enrolled in the KEEPS Cog study was recently completed. The KEEPS Cog study is the first multi-site, randomized, placebo-controlled, double-blind, parallel-group design clinical study that will address major HT-related issues raised by WHI and WHIMS. Specifically, the KEEPS Cog study will compare the differential efficacy of CEE and transdermal estradiol on a comprehensive battery of cognition and mood measures, sensitive to cognition changes associated with HT in perimenopausal and recently-postmenopausal women. The hypothesis of the KEEPS Cog study is that, compared to CEE, treatment with transdermal estradiol will enhance cognitive function of healthy peri- and early menopausal women (i.e., decreased rate of cognitive decline or enhanced rate of cognitive improvement compared to placebo-treated group). The battery of cognitive tests and mood inventories are administered at baseline and months 18, 36, and 48 during treatment (see Table 1). The evaluations at months 18 and 48 will characterize the effects of estrogen therapy alone; thus testing will occur while subjects are not taking the progesterone challenge. The evaluation at month 36 will be timed to assess the effects of estrogen plus progesterone, and will be performed between days 6 and 12 of the progesterone challenge.

Ultimately, the KEEPS Cog study will build upon the knowledge gained by WHIMS, addressing unresolved questions of pivotal clinical significance that need to be
systematically evaluated in well-designed human studies. The study will provide insight into: 1) whether there is cognitive benefit or harm associated with HT administered during the ‘critical period’ (as opposed to late postmenopausal HT investigated in WHIMS), 2) whether there are differential cognitive effects of various estrogen formulations (CEE vs. estradiol), 3) if there is a preferred route of estrogen administration (oral vs. transdermal), 4) whether a cyclic micronized progestin is associated with cognitive benefit, and 5) what are the most sensitive psychometric measures to characterize potential effects of estrogen on cognition and mood.

Given the adverse findings of WHIMS in postmenopausal women and the fact that HT is still approved for the treatment of menopausal symptoms commonly experienced by younger perimenopausal women, it is critical that the potential cognitive effects of HT, both beneficial and adverse, be identified in women undergoing menopausal transition. The KEEPS Cog study will be the first clinical study to characterize the differential effects of oral CEE and transdermal estradiol on cognitive function of perimenopausal women. Additionally, results of the KEEPS Cog study will provide pivotal data and an exclusive opportunity for future studies to follow the KEEPS cohort over an extended period of 20–25 years to determine whether HT initiated during the perimenopausal period could delay or preferably prevent future development of neurodegenerative diseases like Mild Cognitive Impairment (MCI) and AD. Bhavnani [27] has argued that the WHI was not a true primary prevention study given the advanced age and prevalence of obesity among its participants, and the likelihood that many were beyond the opportunity to ‘prevent’ disease. A follow-up to the KEEPS Cog study would help clarify this important question.

Recent Studies Supporting the use of Hormone Therapy during the Menopausal Transition

Recent evidence from two large studies [28{Shao, 2012 #9523}] examining effects of long-term HT, offer additional support for a protective effect of HT against cardiovascular outcomes and AD. The Danish Osteoporosis Prevention Study is an open label randomized trial comprised of 1,006 women over ten years. In this trial, investigators reported that women randomly assigned to estradiol therapy vs. placebo had a significantly reduced risk of mortality, heart failure, and myocardial infarction, without any increase in risk of cancer, venous thromboembolism (VTE) or stroke. Unlike the WHI, participants were young (mean age 49.7 years), healthy (mean BMI of 25.2 and BP 130/81 mm Hg), and recently menopausal (mean time since menopause approx. seven months). After ten years of randomized treatment, participants were followed for another six years, during which time the reduction in primary vascular outcomes was still present and not associated with an increase in any cancer.

A recent analysis of the population-based Cache County Study comprised of 1,768 women examined whether the association of HT with AD varies with timing or type of HT formulation. Investigators reported that women who used HT within 5 years of menopause had a 30% reduced risk for developing AD in later life, especially if the duration of use was 10 years or longer. Moreover, while results suggest that HT may be beneficial if taken near menopause, HT (particularly opposed HT formulations) initiated in later life may be associated with increased AD risk. These findings offer strong support for a beneficial effect of HT on cardiovascular outcomes and protection from AD if initiated during the menopausal transition.

Thus, many in the scientific community agree that while the risk of cardiovascular events and dementia associated with HT has been clarified for women over the age of 65, numerous
questions remain including the ‘critical period’ hypothesis, influence of HT formulation and route of delivery, and whether HT may be useful for primary prevention of dementia [29].

**Conclusion**

Findings of the KEEPS and KEEPS Cog have the potential to affect clinical care practices and health decisions for many millions of women, particularly in regard to disease prevention [14]. If there proves to be a window of time in the early postmenopause when initiation of long-term HT has a net beneficial effect, then women can feel reassured that treatment of their menopausal symptoms when they are likely to be the most severe, can concurrently protect their vascular system, reduce bone reabsorption, or even elicit neuroprotection from dementia in late life.

**REFERENCES**


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Table 1
Cognitive Battery for Kronos Early Estrogen Prevention Study-Cognitive and Affective Study (KEEPS-CA)

<table>
<thead>
<tr>
<th>DOMAIN OR CONSTRUCT MEASURE</th>
<th>NEUROPSYCHOLOGICAL MEASURE</th>
<th>Included in WHIMS(^#) or WHISCA(^#)</th>
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<tbody>
<tr>
<td>INTELLIGENCE: Baseline only</td>
<td>Primary Mental Abilities(^*)-Vocab(^{152,153})</td>
<td>X</td>
</tr>
<tr>
<td>GLOBAL COGNITION:</td>
<td>Modified Mini-Mental State Exam(^{154})</td>
<td>X</td>
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<tr>
<td>VERBAL AND VISUAL MEMORY:</td>
<td>California Verbal Learning Test-2(^#)(^{155})</td>
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<tr>
<td></td>
<td>NYU Paragraph Recall Test(^{156})</td>
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<tr>
<td></td>
<td>Prospective Memory Test(^{152,153})</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Benton Visual Retention Test(^{157})</td>
<td>X</td>
</tr>
<tr>
<td>LANGUAGE:</td>
<td>Controlled Oral Word Association Test FAS/Animals/Fruits/Vegetables(^{158})</td>
<td>X</td>
</tr>
<tr>
<td>ATTENTION AND EXECUTIVE FUNCTION:</td>
<td>Trail Making Test version A &amp; B(^{158})</td>
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</tr>
<tr>
<td>Divided Attention</td>
<td>Stroop Test (Golden Version)(^{159})</td>
<td>X</td>
</tr>
<tr>
<td>Selective Attention</td>
<td>Letter-Number Sequencing WMS-3(^{160})</td>
<td>X</td>
</tr>
<tr>
<td>Auditory Working Memory:</td>
<td>Digit Span WMS-3(^{160})</td>
<td>X</td>
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<tr>
<td>Visual Working Memory:</td>
<td>Digit Symbol(^{161})</td>
<td>X</td>
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<tr>
<td>COMPUTERIZED COGNITIVE TESTS:</td>
<td>3D Mental Rotation(^{162})</td>
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<td>Visual Spatial</td>
<td>Visual Sensitivity Test(^{162})</td>
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<td>Vigilance/Divided Attention</td>
<td>Stroop Test(^{162})</td>
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<td>Selective Attention</td>
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<tr>
<td>MOOD:</td>
<td>Profile of Mood States(^{163})</td>
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<tr>
<td></td>
<td>Beck Depression Inventory(^{164})</td>
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<td></td>
<td>Brief Patient Health Questionnaire(^{165})</td>
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<tr>
<td>SUBJECTIVE MEMORY COMPLAINTS:</td>
<td>Memory Function Questionnaire(^{166})</td>
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\(^{\#}\) Women’s Health Initiative Memory Study\(^{1–4}\)

\(^{\#\#}\) Women’s Health Initiative Study of Cognitive Aging\(^{152,167}\)

\(^{*}\) Administration to match Resnick et al.’s WHISCA battery\(^{152}\)