Prevention of Alzheimer's Disease in Women: Risks and Benefits of Hormone Therapy - Continuation of: "The Kronos Early Estrogen Prevention Study (KEEPS)"

NCT03718494

October 23, 2020

Prospective Observational Study

Cleaned 6-27-17

Modified 10/23/2020

FULL PROTOCOL TITLE

Prevention of Alzheimer's Disease in Women: Risks and Benefits of Hormone Therapy - Continuation of "The Kronos Early Estrogen Prevention Study (KEEPS)"

Study Chairman or Principal Investigator:

Kejal Kantarci, MD
Professor, Radiology

Supported by:

The National Institute of Aging 1RF1AG057547-01
Alzheimer's Drug Discovery Foundation

Version 11

10/13/2020

TABLE OF CONTENTS

STUDY TEAM ROSTER

Kent R. Bailey, PhD
Health Sciences Research
Biomedical Statistics and Informatics
[redacted]
Assistant: [redacted]

Kimberly J. Bailey
Psychometrist
Mayo L- 11
[redacted]

Dustin B. Hammers, Ph.D.,
ABPP(CN)Board Certified in Clinical Neuropsychology
Associate Editor, *Developmental Neuropsychology*Grand Rounds Editor, *The Clinical Neuropsychologist*Past-Chair, APA Committee on Rural HealthAssociate Professor, Department of Neurology
Center for Alzheimer's Care, Imaging & Research
University of Utah
650 Komas Dr., Ste 106A
Salt Lake City, UT 84108
[redacted]Marcelle Cedards
[redacted]

Dr. Howie Rosen

675 Nelson Rising Lane 191F, San Francisco, CA 94158

[redacted]

[redacted]

N. Maritza Dowling, PhD
George Washington University
School of Nursing
[redacted]

Julie A. Fields, PhD, LP
Neurocognitive Disorders
[redacted]

Carey E. Gleason, PhD
Madison VA GRECC (Room D4211)
2500 Overlook Terrace
Madison, WI 53705
[redacted]

Carola Ferrer Simó

Gleason Lab Associate Research SpecialistGRECCUniversity of Wisconsin - Madison VA Hospital, Madison, WIc: [redacted]
[redacted]

Paul N. Hopkins, MD, MSPH
Professor of Internal Medicine
Cardiovascular Genetics Research
417 Wakara Way, Room 2124
Salt Lake City, UT 84108
Office: [redacted]

Kejal Kantarci, MD, MS
Consultant
Department of Radiology
Division of Neuroradiology
Professor of Radiology
Direct: [redacted]
Secretary 507-284-9770
Fax: 507-284-9778
Kantarci.Kejal@mayo.edu

Ekta Kapoor, MBBS, FACP
Assistant Professor of Medicine
Women's Health Clinic (Division of General Internal Medicine)
Division of Endocrinology, Metabolism and Nutrition
[redacted]

June Kendall-Thomas
Clinical Research Coordinator
Radiology Research
[redacted]

Timothy Lesnick
Health Science Research – Biomedical
Harwick 7
[redacted]

Rogerio Lobo
[redacted]

Val Lowe, MD
Radiology - Nuclear Medicine
[redacted]

Mike Malek Ahmadi
[redacted]

JoAnn E. Manson, MD, DrPH
Chief, Division of Preventive Medicine
Brigham and Women's Hospital
Professor of Medicine and the
Michael and Lee Bell Professor of
Women's Health
Harvard Medical School
900 Commonwealth Avenue, 3rd fl
Boston, Massachusetts 02215
[redacted]

Virginia M. Miller, PhD
Surgical Research
[redacted]

Georges Naasan
[redacted]

Lubna Pal, MBBS, MS. FACOG, FRCOG (UK)
Professor, Interim Section Chief, Division of Reproductive Endocrinology & Infertility
Fellowship Director, Reproductive Endocrinology & Infertility
Director, Yale REI Program for Polycystic Ovarian Syndrome
Director, Yale REI Menopause Program
Associate Chair for Education
Department of Obstetrics, Gynecology &

Reproductive Sciences
Yale School of Medicine.
333 Cedar Street, P.O. Box 208063
New Haven, CT 06510.
[redacted]

Miguel Pampaloni
[redacted]

Denise Reyes
Medical Imaging
Opus 2 – 163
[redacted]

Hugh Taylor
[redacted]

Samantha M. Zuk
Medical Imaging Analyst
Opus 2 – 147

PRÉCIS

Study Title
Prevention of Alzheimer's Disease in Women: Risks and Benefits of Hormone Therapy - Continuation of " The Kronos Early Estrogen Prevention Study (KEEPS)"

Objectives
The objectives are to assess the long-term risks and benefits of menopausal hormone therapy (mHT) on Alzheimer's disease (AD) pathophysiology, cerebrovascular, cognitive, and mood health in women treated with transdermal 17β-estradiol (tE2) or oCEE compared to placebo within three years of menopause, which is considered to be the "critical window" for mHT.

Design, Outcomes, Interventions, and Duration
This project is proposed as a continuation to the Kronos Early Estrogen Prevention Study (KEEPS), a nationwide, multi-center, randomized blinded study of mHT in recently menopausal women.

The current investigation will include assessments conducted over 3-4 visits, consisting of chemistries, questionnaires, neurocognitive testing, and brain imaging.

Interventions and Duration

-
    - Explanation of the study and confirmation of eligibility
    - Measurement of height, weight, waist and hip circumference, blood pressure
    - and pulse
    - Review of medical history
    - Blood tests
    - Questionnaires about general health and well-being, COVID-19 questionnaire quality of life, sleep, emotional health and mood and pregnancy history.
    - Neurocognitive tests
    - Brain magnetic resonance imaging (MRI)
    - Brain positron emission tomography (PET): F-18 Florbetapir PET (all sites) and F-18 AV-1451 PET (only at the Mayo Clinic & BAI site)
    - All tests and procedures will take about 9 hours total to complete.

Participants will be on study until all of the following are completed: medical assessment, questionnaires, blood work, neurocognitive studies, , brain MRI, brain PET.

Sample Size and Population
Our targeted enrollment is 492 (n=83 at the Mayo Clinic) participants across eight sites; 13 years post-randomization (4 years of study drug and 9 years after the end of study drugs).

Women who enrolled in the KEEPS study at 8 sites will be invited to participate (total n=688; Mayo Clinic n=118). In order to reduce the costs but retain as many participants as possible, we merged the cohorts in two sites by taking advantage of the proximity of geographic location. Columbia University will be recruiting the Einstein Montefiore Center's participants; therefore 7 sites including Mayo Clinic Rochester will be participating.

1. STUDY OBJECTIVES

1.1 Primary Objectives

To assess effects of menopausal hormone therapy and normal aging on cognitive performance and imaging markers of brain structure in women approximately thirteen years after enrolling in the KEEPS trial. KEEPS participants were randomized to oral or transdermal estrogen treatments or placebo within three years of menopause. This is a follow up study of these women approximately thirteen years after randomization (9 years after study completion.) **No treatments are given as part of this study**; any current hormonal treatments are by choice and prescribed by the participant's personal physician.

Aim 1: Determine the differences in Aβ and tau pathology of AD, cerebrovascular lesions and brain structure in women who were treated with either oCEE or tE2 compared to placebo during early post menopause.

Hypothesis 1: Aβ, tau, cerebrovascular lesions and brain structure are different in women who were treated with tE2 vs. placebo and oCEE vs. placebo during early post menopause, modified by the *APOE* ε4 status.

Aim 2: Determine the differences in longitudinal changes in cognitive performance and mood in women who were treated with either oCEE or tE2 compared to placebo within three years of menopause.

Hypothesis 2: The longitudinal change in cognitive performance and mood over 13 years in women treated with one of the two active estrogen formulations (tE2 or oCEE) will differ from longitudinal cognitive and mood changes occurring in women who received placebo, modified by the *APOE* ε4 status.

Aim 3: To determine the association between imaging markers investigated in Aim 1 and the change in cognitive performance investigated in Aim 2 eight to nine years after completing KEEPS trial participation.

Hypothesis 3: Higher Aβ, tau and WMH load, and smaller regional brain volumes are associated with a longitudinal decline in cognitive performance and mood outcomes since randomization.

2. BACKGROUND AND RATIONALE

2.1 Alzheimer's disease represents a public health crisis, especially for women

Women represent approximately ⅔ of the five million patients with AD dementia. At the age of 65, a woman's lifetime risk for AD is nearly double that for a man, 17.2% compared to 9.1%.[1] While it remains imperative to develop effective AD treatments, the prevailing sentiment is that initiating treatment *after* the onset of clinical symptoms may be "too little, too late" to alter disease course,[2,3] necessitating a shift toward AD prevention in individuals who are at-risk. Thus, there is an urgent need for effective *prevention strategies* to address the suffering and untenable costs associated with AD. How menopausal hormone therapies (mHT) influence the risk of AD remains an area of controversy. As the number of postmenopausal women in the world is projected to be ~1.1 billion in less than a decade from today,[4] and that mHT is prescribed to relieve symptoms of menopause, it is critical that this controversy be addressed in rigorous manner. The results of the proposed research will address this controversy by applying principles of pharmacogenomics with state-of-the art imaging and cognitive testing in postmenopausal women whose hormonal usage and cardiovascular risk factors have been documented for over 10 years. The outcomes would provide evidence for a potential preventive

strategy for women at risk.

2.2 The KEEPS Continuation represents a singular opportunity

The KEEPS, and its ancillary KEEPS Cognitive and Affective Study (KEEPS-Cog) recruited women from 2005 to 2008; all participants were 6 to 36 months past their last menses (age=42-58). The goals of this randomized, double-blinded, placebo-controlled trial was to investigate the cardiovascular, cognitive, and mood effects of two forms of mHT (tE2 and oCEE) administered proximal to the menopausal transition to women whose cardiovascular risks were minimal.[5] In the Women's Health Initiative (WHI) and its Memory Study (WHIMS), oCEE and medroxyprogesterone acetate initiated decades past the menopausal transition, increased the risk of dementia.[6] Since the WHI, prescribing practices have changed such that there are a wide variety of mHTs used to alleviate menopausal symptoms. Whether alternative formulations of mHT, such as tE2 used in KEEPS, can preserve neuronal integrity and decrease the risk of dementia when administered early in menopause remains controversial.[7-14] Our pilot data suggest possible differential effects for two mHT formulations. The inclusion of women who were at higher risk for cardiovascular disease and well past their menopausal transition were considered major design flaws of the WHI study[15] and WHIMS,[16,17] respectively. The KEEPS and KEEPS-Cog were designed to address these limitations, and to determine whether lower doses and different formulations of mHT could reduce the risk of cognitive decline and mood changes that occur during menopausal transition, but only if initiated shortly after menopause during the "*critical window*"; that is, a time-point that would be consistent with a preventive strategy.[18] Women enrolled in the KEEPS are now ~13 years past their randomization to either oCEE or tE2 or placebo, and ~9 years past the termination of study treatment. They are an ideal cohort to investigate potential preventive aspects of mHT as their mHT use was carefully monitored during the clinical trial. Follow-up assessments in the KEEPS Continuation Study will occur at an age (median age=65), when Aβ abnormalities are most likely to dissociate healthy from early AD-related imaging biomarker changes.[19] ***Thus, participants of the KEEPS Continuation Study are at an ideal age to determine the long-term effects of two different formulations of mHT on preclinical AD pathophysiology.*** The consequences of Aβ deposition during early menopausal years are not fully understood, and effectiveness of early mHT for preventing AD-related pathology in the long-term remains unclear. However, reducing Aβ deposition through Aβ-modifying therapies is a widely accepted strategy for preventing AD, and clinical trials are underway in cognitively normal individuals with high Aβ deposition on PET,[2] and in *APOE* ε4 carriers.[20] Therefore, the ability to leverage an identified cohort of women who are *APOE* genotyped, and were exposed soon after menopause to clinically relevant formulations of mHT represents an opportunity not to be missed. Moreover, we are well positioned to investigate the interplay of mHT, cerebrovascular disease, and longitudinal change in cognitive function. A recent examination of records from nearly 500,000 women found that estradiol mHT was associated with lower mortality from vascular dementia (VaD) and AD,[21] and the effect was pronounced for VaD, hinting at the underlying mechanisms of cognitive actions.[21]As discussed in the Funding Opportunity Announcement (FOA), there is a great need to clarify *both* risk and protective exposures in midlife. ***Among the FOA research objectives, the KEEPS Continuation most clearly fulfills the proposal's stated goal to 'test whether putative risk or protective factor are truly causal.'*** Follow-up evaluation of this group of women, whose midlife mHT use and cardiovascular status were well-characterized, provides a singular opportunity to clarify the long-term effects of two formulations of mHT. For women considering mHT, the findings can provide critical insights, guiding their healthcare decisions.

2.3 Estrogens' effects on neurobiology of AD – Importance of the timing of exposure

There is compelling evidence that estrogens influence development of AD neuropathology. Estrogens reduce inflammatory responses,[22,23] especially to Aβ,[24] improve CSF clearance of insoluble Aβ,[25] while increasing expression of non-toxic soluble Aβ,[26] increase synaptogenesis and dendritic spine density in the hippocampal CA1 field,[27,28] and prefrontal cortex,[29,30] and exert

antioxidant effects.[31] In addition, estrogens modulate metabolic function;[32] in particular, regulation of brain mitochondrial glucose transport and glycolysis.[33] However, estrogen's effects may depend on the underlying health of the metabolic system. Brinton [34,35] proposed the concept of a "healthy-cell bias," which suggests that the health of the neuronal substrate will influence the effects of estrogen exposure. Specifically, estrogens increase mitochondrial respiration and ATP generation in healthy neurons, while protecting cells by improving tolerance for calcium influx and increasing antioxidant actions.[36-38] In aged or diseased cells, in which calcium homeostasis is disrupted, estrogen-induced calcium influx becomes deleterious to neurons.[34] Consistent with this supposition, Espeland et, al, recently reported that women with diabetes (age >65) randomized to oCEE in WHIMS demonstrated twice the risk for dementia,[39] and greater gray matter atrophy [40] compared to women without diabetes who were assigned to placebo. Importantly, non-diabetic women on oCEE did not show a greater risk for dementia than the reference group. Hence, the risks and benefits of mHT on cognitive function and AD pathophysiology may depend on a woman's age, her overall health, and in particular, her metabolic and vascular health.[41]

2.4 Seminal studies – WHIMS, WHISCA, KEEPS and ELITE

Two Women's Health Initiative (WHI) ancillary studies, the WHI Memory Study (WHIMS) and WHI Study of Cognitive Aging (WHISCA) found that both opposed and unopposed oCEE were associated with adverse cognitive effects,[16,42-45] and no mood benefits[44,45] when initiated in women age 65 or older. Both treatment conditions were associated with greater brain atrophy than placebo.[46] To clarify the importance of the age at which mHT was initiated, i.e., proximity to menopause, WHI scientists examined the cognitive function of women enrolled in the WHI trial between the ages of 50 and 55 and found no evidence of cognitive benefit or harm more than a decade after mHT was initiated.[47] The KEEPS and its ancillary KEEPS-Cog, and the Early vs. Late Intervention with Estradiol (ELITE) trials were launched in order to address remaining controversies. In KEEPS, mHT was initiated close to the age of menopause; whereas ELITE compared women randomized to mHT within six years of menopause to women exposed more than 10 years past menopause.[32] A review of KEEPS and KEEPS-Cog is provided in our **Preliminary Studies**. Cardiovascular findings from ELITE suggested that early but not late intervention slowed atherosclerosis.[33] Interestingly, the ELITE cognitive trial found no difference in the cognitive effects of mHT based on timing of exposure.[48] Neither KEEPS-Cog nor ELITE has reported on ***long-term effects*** of mHT's. In contrast to WHIMS findings, data from the Prospective Epidemiological Risk Factors (PERF) study suggest that younger women (mean age 54.1) randomized to mHT performed better on cognitive outcomes than women treated with placebo, 5 to 15 years after study involvement ended.[49] It should be noted that in addition to differences in timing of mHT, the formulations of hormones differed among studies. In the WHI, the formulations were oCEE with medroxyprogesterone acetate, a synthetic progestogen.[50] In the ELITE trial, oral 17β-estradiol plus micronized progesterone was used.[32] In the KEEPS, two formulations were compared to placebo: oCEE at a lower dose than in the WHI was used (0.45mg/day) and transdermal 17β-estradiol; each paired with a pulsed micronized progesterone.[5] The formulations used in KEEPS are those most commonly used in clinical practice today. Thus, the KEEPS Continuation study will address inconsistencies in findings, clarifying the effects of mHT on AD biomarkers and cerebrovascular contributions and provide the needed information regarding the long -term cognitive effects of mHT treatments used in current clinical practice.

2.5 Understanding the mechanisms of mHT effects on the brain through imaging biomarkers

F-18 Florbetapir PET directly measures the β-amyloid (Aβ) pathology of AD.[51] A positive Aβ PET scan is proposed as a research criterion for preclinical AD.[52,53] Carriers of the *APOE* ε4 allele are at an increased risk of AD dementia; moreover the risk may be higher in women than in men. [54-56] *APOE* ε4 carriers have increased Aβ deposition at an earlier age than *APOE* ε4 non-carriers,

and this difference is more pronounced in women than in men. [57,58] Thus, women who are *APOE* ε4 carriers are at a higher risk for AD-related Aβ deposition and may benefit most from early initiation of preventive interventions. The age of KEEPS participants we plan to recruit for the current project will be between ages 54 to 70 (median age=65). In the population-based Mayo Clinic Study of Aging (MCSA), 18% of the women have a positive Aβ PET scan at this age range. Aβ PET scan positivity is approximately three times higher in women who are *APOE* ε4 carriers (33% positive) compared to *APOE* ε4 non-carriers (11% positive) in this age range. Similarly, in the Longitudinal Baltimore Study of Aging, *APOE* ε4 positivity conferred a threefold risk of accumulating Aβ after adjusting for sex and education.[59] Although estrogens are thought to modify AD risk, there are only limited data on the estrogen effects on Aβ.[60] Effects of mHT on brain morphology have been investigated in cross-sectional observational MRI studies, with varying findings in cognitively normal postmenopausal estrogen users compared to non-users.[21,61-66] As for all observational studies, these imaging studies are subject to "healthy user bias". Contrary to the findings from observational studies, data from WHIMS indicate greater hippocampal atrophy in postmenopausal women who are treated with oCEE at age 65 years and older.[46] In WHIMS, women with low baseline cognitive function and high ischemic WMH burden were more prone to this treatment effect on the hippocampus, suggesting greater vulnerability to mHT-associated atrophy for already compromised brains.[46,67] Furthermore, hippocampal volumes correlated with cognitive function in the treated group, suggesting oCEE induces cognitive impairment through increased brain atrophy.[68] WMHs were associated with baseline blood pressure in WHIMS, and a greater longitudinal increase in WMH occurred in those with higher blood pressure demonstrating the longitudinal blood pressure effects on the ischemic WMH.[69] MRI findings in WHIMS are consistent with the previously reported decline in cognitive function and increased risk of dementia with oCEE in this cohort, and demonstrate that MRI-based measures of brain morphology are robust biomarkers of cognitive function in postmenopausal women. Additionally, there is evidence that WMH load is associated with small vessel disease in the brain.[67,70] Hypertensive renal disease is strongly associated with WMH,[71] and a better control of blood pressure slows WMH progression.[72,73] An association between WMH load and future risk for mild cognitive impairment is established.[74-76] The KEEPS Continuation Study will examine these underlying disease-associated changes, relating to long - term cognitive effects of two formulations of mHT.

2.6 Overall Impact

Findings from the KEEPS Continuation Study hold the potential to alter the clinical practice paradigms related to treatment of menopausal symptoms and prevention of AD. For the more than 4.5 million postmenopausal women in the world and the women currently using mHT, clarifying the long-term effects of different types and formulations of mHT on the brain is critical.

The KEEPS Continuation study will be unique in clarifying inconsistencies in the literature regarding mHT and cognitive health in the following ways: 1) Provide information regarding potential preventive or risk effects of clinically relevant formulations of mHT on AD biomarkers and cerebrovascular lesions; 2) Provide 13 year follow-up of cognitive change in women who initiated mHT early in menopause; 3) Evaluate the efficacy of mHT relative to APOE ε4.

3. STUDY DESIGN

This is a continuation of the Kronos Early Estrogen Prevention Study (KEEPS) (KEEPS; NCT00154180; Mayo Clinic IRB #2241-04-00), a multicenter double blinded, placebo-controlled, randomized trial (NCT00154180) funded by the Kronos Longevity Research Institute, Phoenix, AZ to test the hypothesis that hormone therapy started early in menopause (within the **"window of opportunity**") would slow progression of atherosclerosis as measured by changes in carotid artery intima-medial thickening and coronary arterial calcification. Enrollment in the KEEPS ended in 2008 with the final study visits occurring in late 2012.[78] 727 healthy menopausal women aged 42 to 58 years, all within 36 months from last menses without prior CVD events were enrolled at nine US

academic sites.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

This proposal is developed as a continuation of the Kronos Early Estrogen Prevention Study (KEEPS; NCT00154180; Mayo Clinic IRB #2241-04-00).

This study will involve a single follow-up evaluation of brain imaging and cognitive function of all women who were randomized to mHT or placebo, approximately 13 years post-randomization and nine years after the end of mHT administration phase in KEEPS. All women who were enrolled in KEEPS in the eight sites will be invited to participate. Names and contact information of the participants were retained by site PIs.

Table 1. Study timeline and enrollment plan and assuming 70% participation rate

Site	Year 1		Year 2	Year 3	Year 4	Year 5	Total
Brigham & Women's	Preparation phase		21	21	15	Analysis	**57**
Columbia & Einstein	Preparation	28	42	42	30	Analysis	**142**
Mayo Clinic	Preparation phase		30	30	22	Analysis	**83**
University of San Francisco	Preparation phase		6	19	14	Analysis	**39**
University of Utah	Preparation phase		24	24	16	Analysis	**64**
Banner Alzheimer's Institute	Preparation phase		7	23	16	Analysis	**46**
Yale University	Preparation	10	20	20	11	Analysis	**51**
Total		**38**	**150**	**179**	**125**		**492**

Each woman will be invited to participate by a letter from the study coordinator at each site. For those women expressing interest, the study coordinator will provide a detailed description of the study and those who are willing to participate will be scheduled for study visits at 13-year post-randomization landmark. Information on current use of medications and interim cardiovascular, cerebrovascular, or neurologic disease events will be collected during the clinical visit. Study participants will be scheduled to complete all assessments and tests over three or four visits. Study data will be de-identified and transferred to the Mayo Clinic and UW through secure file transfer protocols. Data will be stored at each institution's data center.

For participants that have moved away from the site in which they originally were enrolled, may participate at one of the participating sites listed that is closest to their new address. Once they have responded that they are willing to participate at a different site, contact information will be given to the new enrolling site in order to communicate with the participant for Consenting, Enrollment and Scheduling Visits.

At Mayo Clinic, 118 women met the inclusion criteria for randomization into the KEEPS trial. This study will recruit these 118 menopausal women for follow-up. Assuming a 70% retention rate, we project that our cohort will consist of approximately 82 subjects.

4.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria in order to participate in this study:

- Participated in the original KEEPS trial
- able to understand study procedures
- willing to sign an authorization of consent in order to participate in this study.

4.1 Exclusion Criteria

Exclusion Criteria for MRI and PET imaging:

- Women who have contraindications to MRI or PET for safety reasons, such as an MRI-incompatible implant or claustrophobia.
- .

4.2 Study Enrollment Procedures

Once IRB approval has been obtained, a standard IRB approved recruitment and informed consent process will be followed. A sample of 118 women who participated in the KEEPS study at Mayo Clinic who meet the inclusion criteria will be considered eligible for the study. Investigators will provide names and contact information for all 118 women who previously participated in the KEEPS study. Participant names, contact information will be recorded directly into the Medidata Rave ™ database (described below) on a secure institutional server at the Mayo Clinic. This server will be accessible only to study staff. A study coordinator will send eligible participants a letter by mail and invite them to participate.

Eligible participants will be asked to call the study coordinator if interested, or to mail a response card indicating no interest in further contact. For interested individuals, the study coordinator will provide a detailed description of what the study involves and will collect information on current use of medication and interim cardiovascular, cerebrovascular, or neurologic disease events. Individuals who meet eligibility criteria will scheduled for a study visit at the Mayo Clinic Clinical Research Unit (CRU) of the Center for Translational Science Activities. Participants will be instructed to fast for 12 hours prior to that visit.

A similar procedure will be followed at the participating sites that will obtain their own IRB approvals to enroll participants.

5. STUDY PROCEDURES

6.1 Schedule of Evaluations

Study participants will be scheduled to complete the following assessments and tests over 3-4 visits:

Medical history will include reproductive history (including pregnancy related disorders such as gestational diabetes or hypertensive disorders), cardiovascular and cerebrovascular symptoms or conditions, and use of medications including mHTs (about 30 min).

Clinical examination will include blood pressure, pulse, height, weight, and waist-hip measurement and examination for the diagnosis of MCI according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria,[96] or dementia according to DSM V. (about 30 min)

Blood will be collected for testing of the lipids, fasting glucose, Vitamin B12 and TSH levels, C-reactive protein, COVID-19 Antibodies, DNA and future testing. Kits will be shipped to the sites and sites will ship all of the samples back to Mayo Clinic in batches (about 30 min).

Questionnaires, assessing menopausal symptoms (Menopause Rating Scale - MRS), and quality-of-life (Utian Quality of Life Scale – UQOL) will be administered. Sleep quality will be assessed using the Pittsburg Sleep Quality Index (PSQI) Profile of Mood States (POMS), . Data will be electronically transferred to UW (about 1 hour).

Neurocognitive testing, including Modified Mini-Mental State Exam, California Verbal Learning Test-II, New York University paragraph recall tests, Benton Visual Retention Test, Subtests from the Wechsler Memory Scale-III (Digit Span and Letter Number Sequencing subtests), Stroop Color Word Test, Trail Making Test A & B, Digit Symbol Coding from the Wechsler Adult Intelligence Scale – 3,, Letter Fluency and Category Fluency tests, Memory Function Questionnaire, Brief Patient Health Questionnaire (about 2 hours)

Brain MRI (about 1 hour)

Brain PET CT (two exams about 2 hours each)

6.2 Description of Evaluations

6.2.1 Screening Evaluation and Consent

Informed consent: Women meeting eligibility criteria by phone screen will be scheduled to report to the Clinical Research Unit (CRU) located at the Charlton Building. The study coordinator will obtain informed consent by Mail in Consent or in person.

6.2.2 Enrollment &Baseline

Initial enrollment visit: After obtaining informed consent, a basic health assessment will be obtained through interview by the study coordinator, including cardiovascular and cerebrovascular symptoms or conditions, menopausal symptoms, and use of medications. Physical measurements will be obtained by CRU staff, including blood pressure, pulse, height, weight, and waist-hip circumference, and a fasting venous blood sample (about 5 tablespoons) will be collected for analysis of lipid panel, fasting glucose, Vitamin B12 and TSH levels, C-reactive protein, DNA and future testing. . with interpretation at the CRU will be obtained. Questionnaires will be administered. The entire visit will take approximately 2 hours.

Baseline Assessments: This is a follow-up study; single tests will be performed.

6.2.3 Follow-up Visits

Visits 2 -4 will include neuropsychological testing, brain MRI and brain PET CT.

Neuropsychological Testing: A comprehensive battery of standardized neuropsychological tests will be administered by an individual trained by personnel overseeing the cognitive Aim (Primary Objective 2) from the University of Wisconsin, Madison, (PI, Dr. Carey Gleason).The neuropsychological tests are administered in the Research Psychometrics Resource at Mayo Clinic's Center for Translational Science Activities (CTSA) under the direction of Dr. Julie Fields.

The battery will consist of tests used in the original KEEPS study.(81). In addition to one global measure (Modified Mini-Mental test), all tests have been shown to load on one of four factors derived from data reduction procedures described previously.(82)

The four domains of cognitive performance will include:

1. Verbal Learning & Memory function using a composite domain z-score from California Verbal Learning Test (CVLT) and New York University (NYU) Paragraph tests
2. Auditory Attention & Working Memory function using a composite domain z-score

from Wechsler Memory Scale-III Letter-Number Sequencing and Digit Span subtests

3. Visual Attention & Perceptual Speed using a composite domain z-score from
4. Benton Visual Retention Test, Trail Making Test parts A and B, Stroop, and Digit Symbol Coding tests.
5. Speeded Language & Mental Flexibility using a composite domain z-score from Letter (FAS) Fluency and Category (Animals, Fruits, Vegetables) Fluency tests.

Brain MRI: This test will be obtained at the Radiology Department imaging facilities at the Charlton North Building. All MRI studies will be performed on a single 3T system (MAGNETOM, Siemens). A second 3T system with similar hardware and software will be identified as a back-up scanner All MRI sequences used for this study will be acquired in a single sitting with an exam time under 45 min. The operating conditions of the scanner are controlled by its software, and will be in strict adherence to the non-significant risk guidelines as determined by the IRB and as defined by the FDA. The entire system is operated by commercially-available, 510(k) cleared software.

Brain PET Imaging: This test will be obtained at the Radiology Department imaging facilities at the Charlton Building.

F-18 Florbetapir PET: After a 50-minute uptake period, the patient will be positioned on the scanner bed with instructions to remain motionless. Each participant will be injected with 10 mCi of ^{18}F-Florbetapir (target dose 370 MBq, range 296 - 444 MBq). A helical CT image will be obtained at 50 minutes after injection of ^{18}F-Florbetapir, followed by a 20-minute PET acquisition consisting of four 5-minute dynamic frames.

F-18 AV-1451 PET: Under a Material Transfer Agreement (MTA) with Avid Radiopharmaceuticals, we have received their AV-1451 radiosynthesis method. We have replicated that method in our PET radiochemistry laboratory to produce AV-1451. We have acquired the regulatory permissions (IND124447; Val Lowe MD) and IRB approval. Participants will receive an intravenous bolus injection of approximately 370 MBq (10 mCi) of ^{18}F- AV-1451. PET/CT image acquisition will include a low dose CT and then a 20-minute PET acquisition starting at 50-60 minutes after injection of ^{18}F-T807 and will be performed as 4, 5-minute frames that will then be summed into one static frame. Serial frames will allow for adjustment in the case of patient motion.

6. SAFETY ASSESSMENTS

Participation in this study may involve some discomforts or risks:

Blood Draws: The problems associated with blood drawing include discomfort from insertion of the needle (common), fainting at or about the time of blood drawing (infrequent), bruising at the site of the blood drawing (infrequent), and a clot or infection at the same site (rare).

Cognitive testing: It is possible that anxiety may result from the neuropsychological testing.

Brain MRI: Individuals with claustrophobia may feel too confined and may not tolerate MRI scanning. If this occurs, the MRI scan will be stopped. Individuals will wear earplugs during the scan to reduce the discomfort from noise by the MRI machine.

PET/CT scan: During PET imaging, participants will be exposed to radiation from x-rays and radioactive materials. The amount of radiation exposure has a low risk of harmful effects.

PET compound: The radioactive mixture will be injected in the study participant's vein (intravenous). This can result in a risk of pain or bruising or infection at the site of the needle stick.

^{18}F-Florbetapir is an agent with specific high affinity for aggregated amyloid similar to other

Thioflavin-T analogs and is non-toxic. This compound is cleared from the body within minutes and no toxic effects have been recorded with doses used in the study. As with any medication, allergic reactions are a possibility.

^{18}F-T807 (or AV-1451, the AVID Radiopharmaceuticals trade name on the FDA IND) drug safety studies have been completed in rats, mice, dogs and non-human primates, and are available in the Investigator's Brochure on File with the FDA. Testing for binding to CNS relevant receptors, potassium channels, metabolism of ^{18}F-T807 in mice, effect on human microsomes and hepatocytes, genetic toxicity, and cytotoxicity in normal and cancer cell lines has been performed.

With respect to neurological assessment, the no observed effect level (NOEL) of ^{18}F-T807 in rats is at least 200 µg/kg (100x MHD, allometrically scaled), the highest dose tested. Thus, ^{18}F-T807 is not expected to induce CNS effects in humans. With respect to respiratory function, the no observed effect level (NOEL) of ^{18}F-T807 in rats is at least 200 ug/kg (100x MHD, allometrically scaled), the highest dose tested. In conclusion, no respiratory effects are expected in humans from ^{18}F-T807. With respect to cardiac function, the NOEL for males and NOEL for females was determined to be 100x MHD (allometrically scaled) on Day 1 and 50x MHD (allometrically scaled) on Day 29. In summary, ^{18}F-T807is not expected to prolong the QT interval or have any untoward cardiovascular effects at the intended clinical dose. ^{18}F-T807 showed the potential for genotoxicity in *in vitro* bacterial reverse mutation assay (Ames test) and chromosomal aberration assays. However, when potential *in vivo* genotoxicity of ^{18}F-T807was evaluated in a rat micronucleus study, ^{18}F-T807 did not increase the number of micronucleated polychromatic erythrocytes at the highest achievable dose level (1600 µg/kg/day for two days). This dose is greater than 750x the intended MHD, and there is no evidence for risk to human subjects at the proposed micro dose.

In summary, single-dose toxicology studies at doses up to 150x MHD and repeat-dose studies up to 50x MHD for one month showed no clinically important effects. ^{18}F-T807was positive in the *in vitro* hERG assay; however, *in vivo* cardiovascular assessments in dogs showed no evidence of QT prolongation. Nonetheless, until sufficient human cardiovascular safety data are available, initial clinical studies will exclude subjects with a history of risk factors for Torsades de Pointes and subjects taking drugs known to prolong the QT interval. Update: Per FDA update May 2020, recent data shows this is no longer a concern as a risk to participants who have a history of risk factors for Torsades de Pointes and subjects taking drugs known to prolong the QT interval.

Human studies of ^{18}F-T807 at Mayo Clinic will be performed under FDA Investigational New Drug (IND) approval. ^{18}F-T807 will be produced for patient use at Mayo Clinic for this study according to cGMP guidelines. We will cross-file our IND submission with the IND approval on file with the FDA as submitted previously by AVID Radiopharmaceuticals (by permission of AVID Radiopharmaceuticals). All studies will also be performed under Mayo Clinic IRB approval and with informed consent for each subject. All subject data will be kept confidential and subjects will be assigned a unique study number known to the study staff to protect their identity as specified by Mayo Clinic IRB guidelines.

Adverse Events: Treatment emergent adverse events were reported in a total of 3 subjects who received a dose of ^{18}F-T807. Two subjects reported headache, and one subject reported diarrhea. All events were mild in severity and not considered related to ^{18}F-T807 administration by the investigator. No serious adverse events were reported for any subject receiving ^{18}F-T807. No consistent or clinically significant changes in vital signs, laboratory values, or ECG results were observed in a completed analysis of 11 subjects.

Radiation Dosimetry: Preliminary radiation dosimetry assessment was performed in three dosimetry subjects. The radiotracer biodistribution among the subjects was consistent and showed rapid hepatobiliary clearance. There were three organs that received estimated doses higher than 0.05

mSv/MBq. The organ that received the largest estimated dose was the upper large intestinal wall (0.107 ± 0.009 mSv/MBq), followed by the small intestine and the liver. The Effective Dose was 0.0248 ± 0.0011 mSv/MBq. This results in an estimated Effective Dose of 9.18 mSv for an anticipated 370 MBq (10 mCi) injection and is comparable to the effective dose of approved ^{18}F-labeled compounds such as fluorodeoxyglucose (FDG) and florbetapir F 18 injection, and consistent with the primate dosimetry studies.

All participants will receive full supportive care while participating in the protocol. Mayo Clinic physicians remain on-call 24 hours per day to respond to any questions regarding subjects health concerns while participating in this protocol.

Participants may choose to discontinue participation in the study at any time.

7.1 Specification of Study Parameters

Unexpected abnormal results on any testing completed as part of participation in this study will be reviewed by the principal investigator team and reported to the participant in a timely manner depending on the urgency and clinical significance of the abnormality.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The Principal Investigator or designee will review all reported side effects up to 8 weeks after the patient's study to assess adverse events. Any adverse event reported to either the principal investigator or his designated research associates by the subject or medical staff caring for the subject will be recorded and the nature and attribution of cause of the event will be discussed. Any new adverse event that is also attributable to the study will be documented as such.

7.3 Adverse Events and Serious Adverse Events

An adverse event includes both, an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. Any new, serious adverse events as described by Common Terminology Criteria for Adverse Events (CTCAE) v3.0 as found at http://ctep.cancer.gov/forms/CTCAEv3.pdf) that are described will be reported to the IRB within 24 hours and the trial will be suspended immediately for review by the IRB. No serious side effects from the research imaging methods in this protocol are conceivable or expected.

7.4 Reporting Procedures

Any adverse event that is so determined, or reported otherwise to either the principal investigators or their designated research associates by the subject or medical staff caring for the subject and which meets the criteria for a new adverse event that is also attributable to the study will be documented as such.

7.5 Follow-up for Adverse Events

The Principal Investigator or designee will review all reported side effects up to 8 weeks after the patient's study (reviews done on a monthly basis) to assess adverse events.

7.6 Safety Monitoring

As noted in 7.2.

7. INTERVENTION DISCONTINUATION

Attributable adverse events will be reported to the IRB and the study suspended for discussion when needed.

8. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The proposed analytical plan will comprise 1) data cleaning and basic statistical analyses to identify potential outliers, assess for normality, and examine variation in each variable; 2) the generation of standard descriptive statistics summarizing the sample and its characteristics; and 3) statistical methods for analyzing each specific aim and hypothesis. For each aim, all calculated *P* values will be 2-sided and $P<.05$ will be considered statistically significant.

9.2 Primary Objective 1

To determine the differences in Aβ, cerebrovascular lesion load and brain structure in postmenopausal women who were treated with one of two mHTs vs. placebo after 13 years post-randomization and 9 years after the end of mHT administration phase.

Primary Hypothesis: Aβ PET SUVR (primary outcome), AV-1451 SUVR, WMH volume and regional cortical thickness (or hippocampal volume) are different in women who were treated with tE2 vs. placebo and oCEE vs. placebo.

Hypothesis 1a: Aβ PET SUVR (primary outcome), AV-1451 SUVR, WMH volume and regional cortical thickness (or hippocampal volume) differences in women who were treated with tE2 vs. placebo and oCEE vs. placebo are modified by the *APOE*e4 status (carrier/noncarrier).

Hypothesis 1b: Aβ PET SUVR (primary outcome), AV-1451 SUVR, WMH volume and regional cortical thickness (or hippocampal volume) differences in women who were treated with tE2 vs. placebo and oCEE vs. placebo during early post menopause are modified by the vascular disease risk at 13 years post-randomization.

Statistical Analysis for Aim 1: We will first summarize the data for Aim 1 using standard descriptive statistics and a variety of plots (histograms, scatterplots) to assess variable distributions. Our outcome measures: Aβ PET SUVR (primary outcome), WMH volume, and regional cortical thickness (or hippocampal volume) for this aim will all be continuous cross-sectional variables, we will use analysis of covariance (ANCOVA) to address the primary hypothesis. Treatment groups (tE2, oCEE, and placebo) will be included as the predictors of interest, coded so that placebo is the reference group. Standard methods (Least Significant Difference, Tukey's Honest Significant Difference, and Scheffé's test) will be used to assess pairwise differences. We will include a set of dummy variables to test and adjust for site (block) effects. The outcome variables will be transformed as necessary to meet regression assumptions underlying the ANCOVAs. These analyses will include baseline age, time from baseline, and APOE ε4 status (as appropriate) as covariates. We will test for interactions of treatment with *APOE* ε4 status through inclusion of two-way interactions in the models, and analyses in separate *APOE* ε4 non-carrier and *APOE* ε4 carrier strata. To address Hypothesis 1a, we will expand the set of covariates to include a vascular disease risk score assessed at the time of mHT/placebo initiation(82) or individual cardiovascular risk factors (systolic and diastolic BP, fasting glucose, smoking history, lipid profile, BMI, waist circumference). Possible interactions with these covariates will be handled in the same way as *APOE* ε4 status. Hypothesis 1b will be treated much the same way, but CVD risk scores will be constructed from data acquired at 13 years after initiation of mHT. Since some of the women in the study might have used mHT after the trial ended, we will perform a series of sensitivity analyses with and without those individuals to assess any important effects and ensure that the final conclusions are robust.

Power for Aim 1: Focusing on Florbetapir PET, our primary outcome variable for Aim 1, we first estimated the standard deviations in three groups of women (all subjects, *APOE* ε4 non-carriers, and *APOE* ε4 carriers) from the Mayo Clinic Study of Aging, a longitudinal study on aging and dementia.[117] We identified women with an age range of 55-70 (mean age=65) similar to our projected sample, who underwent both Aβ PET imaging and *APOE ε4* testing (n=264) Using

these standard deviations as estimates of variability in our projected sample, we estimated the minimum detectable effect sizes in the pairwise comparisons (using Cohen's d) with 80% power.

Table 3. Minimum detectable effect sizes for projected sample size and 80% power.

Comparison	All	*APOE ε4* non-carriers	*APOE ε4 carriers*
oCEE vs. Placebo	0.308	0.358	0.619
tE2 vs. Placebo	0.312	0.355	0.665

These effect sizes fall in the medium (all and *APOE* ε4 non-carriers) to large (*APOE* ε4 carriers) range. In our 84 month KEEPS data, most of our observed Cohen's d values were close to 0.34 (all subjects), 0.37 (*APOE ε4* non-carriers), and 1.18 (*APOE ε4* carriers). We anticipate the effect sizes to be at least this large in the KEEPS Continuation study. Since the minimum detectable effect sizes in **Table 3** are close to or smaller than these values, we anticipate having adequate power to detect pairwise differences. We would expect similar effect sizes for the other outcomes.

9.3 Primary Objective 2

To determine the differences in longitudinal changes in cognitive performance and mood in postmenopausal women who were treated with one of the two mHTs vs. placebo.

Primary Hypothesis 2: Performance on four cognitive factors described in our bi-factor model and on POMS mood indices are different in women who were treated with tE2 vs. placebo and oCEE vs. placebo.

Hypothesis 2a: Cognitive and mood differences in women who were treated with tE2 vs. placebo and oCEE vs. placebo are modified by *APOE*e4 status (carrier/noncarrier).

Hypothesis 2b: Cognitive and mood differences in women who were treated with tE2 vs. placebo and oCEE vs. placebo are modified by the vascular disease risk at 13 years post-randomization.

Statistical Analysis for Objective 2: Analyses for this aim will focus on longitudinal cognitive outcome data collected over six predetermined and unequally spaced time points over a total period of 13 years. We will employ linear mixed-effects (LME) regression models with treatment groups (oCEE and tE2) vs placebo (and their interactions with time) as predictors of cognitive change, and baseline age, *APOE ε4, and* education as control variables as well as in *APOE ε4* carriers and non-carriers separately. Separate models will be estimated for each of the cognitive factor scores and mood outcome measures. The cognitive factor scores include the following four domains: verbal learning and memory; auditory attention and working memory; visual attention and perceptual speed; speeded language and flexibility. The mood outcome will be measured with the Profile of Mood States (POMS). The LME model incorporates covariance structures to account for the correlation between repeated measures across time. The analyses for the LME model will be conducted using the limited information maximum likelihood complete sample approach to missing data. The incorporation of higher order terms in the mixed effect models will also be examined. All models will test and adjust for site effects. The shape of the trajectories across time will be inspected by plots and models will account for possible non-linearity. Random effects will be evaluated by likelihood-ratio ($\chi 2$) tests and fixed effects will be evaluated via F-tests based on Type III sums of squares. Quantile-quantile plots of residuals will also be examined for evidence of significant outliers. As in Aim 1, we will address Hypothesis 2a by adding to the models a vascular disease risk score assessed at the time of mHT/placebo initiation[84] or individual cardiovascular risk factors. Possible interactions with these covariates will be handled in the same way as *APOE* ε4 status. Similarly, for Hypothesis 2b we will derive CVD risk scores from data acquired at 12 years after initiation of mHT. Since some of the

women in the study might have used mHT after the trial ended, we will perform a series of sensitivity analyses with and without those individuals to assess any important effects and ensure that the final conclusions are robust.

Power for Objective 2: Power and sample size estimates to detect significant changes in cognitive function are based on Mayo Clinic pilot data collected over five unequally-spaced observation time points (0,18, 36, 48, and 84 months) with the verbal learning and memory factor as the longitudinal outcome and treatment groups vs. placebo as predictors. We assumed normally distributed continuous outcomes with missing data, two predictors (oCEE and tE2) with regression coefficients in factor score units for rate of change of, respectively, -0.20 and -0.11; a variance of 2.09 for the random intercept, a variance of 0.03 for the random slope, and a correlation between random slope and random intercept terms set to 0.07. Using a two-tailed α=0.05, a total sample size of 492 individuals will achieve a power of 82% to reject the hypothesis for this aim.

Alternative Strategy: We will evaluate our participants for incident MCI and dementia, but we do not expect to have sufficient power to test the differential progression to MCI or dementia in each of the mHT vs. placebo groups. However, we will explore this alternative approach if we have a sufficient number of incident cases.

9.4 Primary Objective 3

To determine the associations between imaging biomarkers investigated in Aim 1, and longitudinal changes in cognitive performance and mood investigated in Aim 2.

Primary Hypothesis 3: Higher Aβ (primary outcome), AV-1451 and WMH load, and smaller regional cortical thickness are associated with a longitudinal decline in cognitive performance since randomization

Statistical Analysis for Objective 3: Repeated cognition and mood measures will be available for time points ranging from trial baseline to 13 years post-baseline; we will use the same cognitive factor scores and POMS as in Aim 2. For each measure in Aim 2, we will consider the values at year 13, the changes in values from baseline to year 13, and predicted annual changes in values at year 13 (slopes). Aβ PET SUVR, WMH, and AD signature thickness and hippocampal volume will only be available at 13 years post-baseline. We will use plots and Pearson or Spearman correlation coefficients, as appropriate given the distributions of the variables involved, to describe and measure associations between cognitive/mood measures and imaging biomarkers. As in the previous aims, we will explore and include site and covariate effects through regression models, transform variables as needed to meet assumptions, and perform sensitivity analyses with and without individuals who continued to use mHT after the mHT administration phase ended.

Power for Objective 3: With 492 individuals and α=0.05, we will have 80% power to detect Pearson correlation coefficients as small as 0.127. In a linear regression, this would be an increase in the model R^2 due to the association of interest as small as 0.016. We should thus have sufficient power to detect small associations.

9. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Case report forms (CRFs) have been developed in paper format and will also be made available as electronic CRF (eCRFs), or data entry screens, using infrastructure from the clinical trial management system Medidata Rave ™ Participant source data will be entered directly or transcribed into the eCRFs or captured on paper forms first and then transcribed into the

corresponding eCRFs. In particular, source data obtained at visit 1, including medical history, current use of medications, vital signs and questionnaires will be captured on paper forms and entered in eCRFs. MR images are stored in the Radiology clinical archiving system, and will be transferred to Mayo Clinic designated secure servers after anonymization. PET images are stored in Nuclear Medicine archiving system and will be transferred to Mayo Clinic designated secure servers after anonymization.

Paper CRF for cognitive and mood data will be stored at sites where data are collected. Study coordinators will enter coded data through a password protected, secure, web-based data portal (e.g., REDCap). All cognitive and mood data will be stored on a HIPAA-compliant, secure University of Wisconsin server. Keys linking identity of participants to coded data will remain at the data collection sites.

10.2 Data Management

Cognitive data will be transcribed into the Cognitive data portal, resulting in an eCRFs. The web-based system will be compliant with 21 CFR (Code of Federal Regulations) Part 11 FDA (Food and Drug Administration), Federal Information Security Management Act (FISMA), and Health Insurance Portability and Accountability Act (HIPAA) requirements. (e.g., REDCap) and will include features to guide data entry accuracy. For example, a reason range for data values will be designated, and an error message generated if values are out of range. Edit checks, electronic queries, and audit trails will be included to ensure accurate and complete data collection and security. https://www.project-redcap.org/

Cognitive data storage and management will occur under the direction of Dr. Gleason, with the assistance of a Master's-level statistician. Coded data will be stored in an R dataset with restricted access. As noted, the code will not be stored on the UW server, and will not be accessible to University of Wisconsin personnel.

Additional participant source data will be entered directly or transcribed into eCRFs using Medidata Rave ™, a remote system featuring advanced capabilities in both electronic data capture and clinical data management. Implemented via the Clinic Trials Management Systems (CTMS) project in 2010 the Medidata Rave ™system is compliant with 21 CFR (Code of Federal Regulations) Part 11 FDA (Food and Drug Administration) requirements. Edit checks, electronic queries, and audit trails are built into the system to ensure accurate and complete data collection and security.

Data storage and management at the Mayo Clinic is under the direction of Dr. Bailey, with the assistance of a Master's-level statistician. Data from Medidata Rave ™will be stored as SAS datasets (in Linux) with accessibility restricted to appropriate study members from the Division of Biomedical Statistics and Informatics in the Department of Health Sciences Research.

10.3 Quality Assurance

In addition to the quality assurance measures implemented at the data entry point, cognitive and mood data will be audited for outliers and inaccuracies through standard data cleaning procedures

Each MRI and PET acquired on a study subject scan is rated on image quality using a standardized grading form with electronic data entry into a database at the Mayo Clinic.

.

10.4 MRI Image Analysis

10.4.1 Pre-processing to Correct Specific Artifacts: Several common forms of image

imperfection can degrade the quality of the MR data we will collect. These are intensity in-homogeneity due to B1 receiver non-uniformity; drifts or discontinuities in gradient calibration over time; and gradient non-linearity. Image pre-processing operations designed to correct these effects are applied to each set of images prior to image processing.

10.4.2 MRI analysis of regional cortical structure: 3D Magnetization Prepared Rapid Acquisition Gradient-Echo (MPRAGE) developed for the multi-center ADNI study by the Mayo ADNI team,[107] will be used to provide optimal tissue contrast for brain morphology and is harmonized across Siemens, GE and Phillips scanners. We will investigate the AD-signature region of interest (ROI) that we previously identified in clinically diagnosed and autopsy-confirmed AD patients and tested for diagnostic reliability and accuracy.[108] We will investigate both hippocampus and dorsolateral prefrontal cortex volumes because preservation of these structures have been observed after estadiol treatment in ovariectomized animal models.[109] Furthermore, the **preliminary data in Figure 6** suggest that dorsolateral prefrontal cortex volume is preserved in the tE2 group compared to placebo seven years after starting mHT in the KEEPS MRI sub-sample. We will use both publically available FreeSurfer 5.3[110,111] and in-house atlas-based ROIs for the analysis of regional volumes adjusted for total intracranial volume (TIV). In addition, we will conduct a non-hypothesis based voxel based morphometry (VBM) analysis using Statistical Parametric Mapping 12 (SPM12),[112] to explore regional differences between the mHT and placebo groups in the entire brain using an unbiased approach.

10.4.3 Segmentation and Quantification of White Matter Hyperintensities (WMH) and identification of infarcts: Fluid attenuated inversion recovery (FLAIR) with TR/TE/TI = 6000/390/2100 ms with a 800 ms long turbo spin echo readout train, 750 Hz/pixel bandwidth with 3mm interleaved images of the whole head will be used. WMH volumes are measured using a semi-automated segmentation algorithm as previously described.[113] Briefly, FLAIR and MPRAGE images are co-registered, and the MPRAGE segmentation on SPM12 is used to create a WM mask to reduce false positives on the WMH segmentation from FLAIR. WMH is segmented using an automated slice-based seed initialization and region growing method. The segmented WMH voxels are multiplied with the WM mask and WMH masks are inspected and edited by a trained analyst (blinded to the treatment status) in order to exclude artifacts from the WMH volume. Infarcts will be identified by the same analyst and confirmed by Dr. Kantarci. The hyperintensities on FLAIR images associated with infarcts are marked and are re-classified as hyperintensities associated with cortical or subcortical infarcts. Hyperintensity associated with infarcts are not included in the WMH volume of individual subjects due to pathophysiologic differences between the two lesions.[114] We do not expect a significant number of infarcts in this cohort.

10.4.4 Diffusion Tensor Imaging (DTI): 2D single-shot gradient echo sequence with TR/TE=6600/86 ms, a 128 x 128 base matrix for 240 x 256 mm FOV, 60 contiguous 2 mm slices yielding 2.2 x 2.2 mm in-plane resolution is used for DTI. Diffusion-weighting gradients will be applied along 48 directions with b=1000 m^2/s and 6 non–diffusion T2 volumes (b = 0 m^2/s). This sequence was developed for the multi-center LEFFTDS and ARFL studies by the MCR team led by Dr. Kantarci and is harmonized across scanners in participating sites. We recently tested and validated a method to process DTI scans and analyze fractional anisotropy (FA) maps and demonstrated that improved DTI registration outperforms Tract-Based Spatial Statistics.[102] We will conduct non-hypothesis based voxel based analysis of FA to explore the WM diffusivity differences between each of the mHT and placebo groups in the entire white matter as previously described.[103]

10.4.5 Resting state (Rs)-fMRI: A T2*-weighted gradient echo–echo planar sequence with TR/TE=2000/27 ms, flip angle 80°; FOV=230 x 230 mm; matrix size: 92 x 92; 3 mm

slices with 2.5 x 2.5 mm in-plane resolution will be used. Subjects are instructed to remain awake with their eyes closed. This sequence was developed for the multi-center LEFFTDS and ARFL studies by the MCR team led by Dr. Kantarci and is harmonized across scanners. The images will be realigned correcting for head motion, and unwarped correcting for susceptibility-by-movement interactions, slice-time corrected, co-registered to T1-weighted image, normalized and smoothed using SPM12. After processing, we will examine connectivity within the default mode network using a seed-based approach, by using the BOLD time series for specific regions as a covariate of interest for whole brain regression analyses.[105,106] Each of the mHT group maps will be compared to placebo groups with SPM12.

10.5 PET Image Analysis

For all image acquisitions, attenuation correction will use either CT or PET transmission data, and reconstruction will use site-specific algorithms and the PET imaging. After anonymization, data will be electronically transferred to the Mayo Clinic secure servers. Quality control procedures will include checks to assure that the protocol has been followed, checking for full brain coverage, and motion assessment across temporal frames. The sequence of temporal frames are co-registered to the first frame of each scan, and both a dynamic image set, as well as a single averaged-frame image set are produced. Quantitative analysis is performed using the fully automated image processing pipeline, previously described in detail.[116] Briefly, a cortical global Aβ PET standardized uptake value ratio (SUVR) is obtained by combining the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus ROI values normalized by the cerebellar ROI of an in-house atlas. A similar Region-based SUVR have been developed for tau-PET.

10.6 Training

Trained, certified technicians, who perform the tests also on a clinical basis, will be administering neurocognitive tests, brain MRI, and brain PET.

10.7 Quality Control Committee

Quality control for each test is maintained by the investigative team overseeing that test. Data quality is reviewed by Mayo Clinic and the Principal Investigators for each project.

10.8 Metrics

See Section 10.3 above.

10.9 Protocol Deviations

Any protocol deviations will be documented in Medidata Rave ™ and reviewed by the KEEPS continuation executive committee quarterly throughout the duration of the study.

10.10 Monitoring

Protocol compliance, consent forms, case report forms, timely entry of data, and quality of data will be monitored quarterly by Kent Bailey PhD, assisted by study coordinators and master's statistician.

10. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol, informed consent document, patient contact materials, and any subsequent modifications will be reviewed and approved by the Mayo Clinic IRB responsible for oversight of the study.

11.2 Informed Consent Forms

Informed consent will be obtained by Mail in Consent after review via telephone contact or in person (between study staff and study candidate) and a signed consent form is required for participation. All participants in KEEPS were English speaking and literate. The consent form describes the purpose of the study, study procedures, and the risks and benefits of participation. The consent form also describes that participation in the study is voluntary and that participants can withdraw at any time. Participants will receive an additional copy of their consent form in the packet mailed to them to keep for their personal records.

11.3 Participant Confidentiality

Data generated by the research study will be kept strictly confidential. Databases and study documents with identifiers will be kept on a secure Mayo network drive accessible only to a subset of the study team. HIPPA requirements will be followed. Subjects will be assigned code numbers. Study identification numbers will be retained from the KEEPS assignment so as to be able to link all longitudinal data from each participant. These identification numbers are not linked to medical records. All data will be reported in aggregate. Subjects will be told prior to this study that their participation is voluntary. Although we do not anticipate that the subjects will experience discomfort in participating, they can choose not to answer any questions that make them uncomfortable. Information will not be released without written permission of the participant.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIA, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

11. COMMITTEES

The Co-PIs Dr. Kejal Kantarci and Dr. Carey Gleason will be responsible for planning, reviewing, coordinating, and directing the project. They will direct and oversee all scientific management, administrative, and financial aspects of the program. They will make changes to the program as deemed necessary in consultation with the Executive Committee that consists of site-PIs and meets once a month over tele-conferences.

12.1 Day-to-Day Management at the Mayo Clinic

The Ekta Kapoor MD, who is a women's health specialist is responsible for recruitment, screening, consent and enrollment of study participants, coordination and completion of clinical research testing. Julie Fields PhD, LP is responsible for management and oversight of neurocognitive testing. Kent Bailey PhD is responsible for database management and statistical analyses.

Site- PIs are responsible for the day-to-day administration of the protocol at their sites, including data collection, and transfer.

12.2 External Advisory Committee

An external Advisory Committee will review productivity, allocation of funds, and other issues that may arise in relationship to progress. This Committee may recommend changes needed for the research direction/emphasis. Members of this Committee were selected specifically for their leadership activities, for their clinical and research activities related to the overarching theme of the KEEPS continuation. Members of this committee include:

S. Mitchell Harman MD (chair- PI of the original KEEPS);
Susan Resnick PhD;
Eric Reiman MD;
Clifford Jack MD;
Pauline Maki PhD;
Nannette Santoro MD; and

Frederick Naftolin MD.

The advisory committee will meet over teleconferences and an in-person meeting with key personnel

12. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Executive Committee. All publications will be made public through NIHMS.

13. REFERENCES

1. Aenlle KK, Kumar A, Cui L, Jackson TC, Foster TC. Estrogen effects on cognition and hippocampal transcription in middle-aged mice. *Neurobiol Aging* 2007
2. Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci* 1990;10:1286-1291.
3. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci* 1990;10:4035-4039.
4. Gibbs RB. Estrogen and nerve growth factor-related systems in brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. *Ann N Y Acad Sci* 1994;743:165-196; discussion 197-169.
5. Gibbs RB, Aggarwal P. Estrogen and basal forebrain cholinergic neurons: Implications for brain aging and alzheimer's disease-related cognitive decline. *Horm Behav* 1998;34:98-111.
6. Luine VN. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp Neurol* 1985;89:484-490.
7. Markowska AL, Savonenko AV. Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. *J Neurosci* 2002;22:10985-10995.
8. Green PS, Gridley KE, Simpkins JW. Nuclear estrogen receptor-independent neuroprotection by estratrienes: A novel interaction with glutathione. *Neuroscience* 1998;84:7-10.
9. Green PS, Simpkins JW. Neuroprotective effects of estrogens: Potential mechanisms of action. *Int J Develop Neurosci* 2000;18:347-358.
10. Greene RA. Estrogen and cerebral blood flow: A mechanism to explain the impact of estrogen on the incidence and treatment of alzheimer's disease. *Int J Fertil Womens Med* 2000;45:253-257.
11. Resnick SM, Maki PM, Golski S, Kraut MA, Zonderman AB. Effects of estrogen replacement therapy on pet cerebral blood flow and neuropsychological performance. *Horm Behav* 1998;34:171-182.
12. Chen S, Nilsen J, Brinton RD. Dose and temporal pattern of estrogen exposure determines neuroprotective outcome in hippocampal neurons: Therapeutic implications. *Endocrinology* 2006;147:5303-5313.
13. Jaffe AB, Toran-Allerand CD, Greengard P, Gandy SE. Estrogen regulates metabolism of alzheimer amyloid beta precursor protein. *J Biol Chem* 1994;269:13065-13068.
14. Nilsen J, Chen S, Irwin RW, Iwamoto S, Brinton RD. Estrogen protects neuronal cells from amyloid beta-induced apoptosis via regulation of mitochondrial proteins and function. *BMC Neurosci* 2006;7:74.
15. Resnick SM, Metter EJ, Zonderman AB. Estrogen replacement therapy and longitudinal decline in visual memory. A possible protective effect? *Neurology* 1997;49:1491-1497.
16. Rice MM, Graves AB, McCurry SM, Gibbons LE, Bowen JD, McCormick WC, Larson EB. Postmenopausal estrogen and estrogen-progestin use and 2-year rate of cognitive change in a cohort of older japanese american women: The kame project. *Archives of Internal Medicine* 2000;160:1641-1649.

17. Steffens DC, Norton MC, Plassman BL, Tschanz JT, Wyse BW, Welsh-Bohmer KA, Anthony JC, Breitner JC. Enhanced cognitive performance with estrogen use in nondemented community-dwelling older women. *J Am Geriatr Soc* 1999;47:1171-1175.
18. Grodstein F, Chen J, Pollen DA, Albert MS, Wilson RS, Folstein MF, Evans DA, Stampfer MJ. Postmenopausal hormone therapy and cognitive function in healthy older women. *J Am Geriatr Soc* 2000;48:746-752.
19. Matthews K, Cauley J, Yaffe K, Zmuda JM. Estrogen replacement therapy and cognitive decline in older community women. *J Am Geriatr Soc* 1999;47:518-523.
20. O'Hara R, Schroder CM, Bloss C, Bailey AM, Alyeshmerni AM, Mumenthaler MS, Friedman LF, Yesavage JA. Hormone replacement therapy and longitudinal cognitive performance in postmenopausal women. *Am J Geriatr Psychiatry* 2005;13:1107-1110.
21. Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of alzheimer's disease in women. *Am J Epidemiol* 1994;140:256-261.
22. Szklo M, Cerhan J, Diez-Roux AV, Chambless L, Cooper L, Folsom AR, Fried LP, Knopman D, Nieto FJ. Estrogen replacement therapy and cognitive functioning in the atherosclerosis risk in communities (aric) study. *Am J Epidemiol* 1996;144:1048-1057.
23. Waring SC, Rocca WA, Petersen RC, O'Brien PC, Tangalos EG, Kokmen E. Postmenopausal estrogen replacement therapy and risk of ad: A population-based study. *Neurology* 1999;52:965-970.
24. Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JC, Cache County Memory Study I. Hormone replacement therapy and incidence of alzheimer disease in older women: The cache county study.[see comment]. *JAMA* 2002;288:2123-2129.
25. de Moraes SA, Szklo M, Knopman D, Park E. Prospective assessment of estrogen replacement therapy and cognitive functioning: Atherosclerosis risk in communities study. *Am J Epidemiol* 2001;154:733-739.
26. File SE, Heard JE, Rymer J. Trough oestradiol levels associated with cognitive impairment in post-menopausal women after 10 years of oestradiol implants. *Psychopharmacology (Berl)* 2002;161:107-112.
27. Fillenbaum GG, Hanlon JT, Landerman LR, Schmader KE. Impact of estrogen use on decline in cognitive function in a representative sample of older community-resident women. *Am J Epidemiol* 2001;153:137-144.
28. Petitti DB, Crooks VC, Chiu V, Buckwalter JG, Chui HC. Incidence of dementia in long- term hormone users. *Am J Epidemiol* 2008;167:692-700.
29. Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, Melton LJ, 3rd. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 2007;69:1074-1083.
30. Hogervorst E, Williams J, Budge M, Riedel W, Jolles J. The nature of the effect of female gonadal hormone replacement therapy on cognitive function in post-menopausal women: A meta-analysis. *Neuroscience* 2000;101:485-512.
31. LeBlanc ES, Janowsky J, Chan BK, Nelson HD. Hormone replacement therapy and cognition: Systematic review and meta-analysis. *Jama* 2001;285:1489-1499.
32. Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: Effects on cognitive function and dementia. *JAMA* 1998;279:688-695.
33. Craig MC, Maki PM, Murphy DG. The women's health initiative memory study: Findings and implications for treatment. *Lancet Neurol* 2005;4:190-194.
34. Sherwin BB, Henry JF. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: A critical review. *Front Neuroendocrinol* 2008;29:88-113.
35. Joffe H, Hall JE, Gruber S, Sarmiento IA, Cohen LS, Yurgelun-Todd D, Martin KA. Estrogen therapy selectively enhances prefrontal cognitive processes: A randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause* 2006;13:411-422.
36. Phillips SM, Sherwin BB. Effects of estrogen on memory function in surgically menopausal

women. *Psychoneuroendocrinology* 1992;17:485-495.
37. Schmidt R, Fazekas F, Reinhart B, Kapeller P, Fazekas G, Offenbacher H, Eber B, Schumacher M, Freidl W. Estrogen replacement therapy in older women: A neuropsychological and brain mri study. *J Am Geriatr Soc* 1996;44:1307-1313.
38. Shumaker SA, Reboussin BA, Espeland MA, Rapp SR, McBee WL, Dailey M, Bowen D, Terrell T, Jones BN. The women's health initiative memory study (whims): A trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. *Control Clin Trials* 1998;19:604-621.
39. Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R, Dailey M, Freeman R, Hays J. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's health initiative memory study. *Jama* 2004;291:2959-2968.
40. Espeland MA, Tindle HA, Bushnell CA, Jaramillo SA, Kuller LH, Margolis KL, Mysiw WJ, Maldjian JA, Melhem ER, Resnick SM. Brain volumes, cognitive impairment, and conjugated equine estrogens. *J Gerontol A Biol Sci Med Sci* 2009
41. Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass ML, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D. Effect of estrogen plus progestin on global cognitive function in postmenopausal women: The women's health initiative memory study: A randomized controlled trial. *Jama* 2003;289:2663-2672.
42. Shaywitz SE, Naftolin F, Zelterman D, Marchione KE, Holahan JM, Palter SF, Shaywitz BA. Better oral reading and short-term memory in midlife, postmenopausal women taking estrogen. *Menopause* 2003;10:420-426.
43. Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Fillit H, Stefanick ML, Hendrix SL, Lewis CE, Masaki K, Coker LH. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's health initiative memory study. *Jama* 2004;291:2947-2958.
44. Henderson VW, Espeland MA, Hogan PE, Rapp PR, Stefanick ML. Prior use of hormone therapy and incident alzheimer's disease in women's health initiative study. *Neurology* 2007;68:A205.
45. Asthana S, Brinton RD, Henderson VW, McEwen BS, Morrison JH, Schmidt PJ. Frontiers proposal. National institute on aging "bench to bedside: Estrogen as a case study". *Age (Dordr)* 2009;31:199-210.
46. Henderson VW, Benke KS, Green RC, Cupples LA, Farrer LA. Postmenopausal hormone therapy and alzheimer's disease risk: Interaction with age. *J Neurol Neurosurg Psychiatry* 2005;76:103-105.
47. Maki PM. Hormone therapy and cognitive function: Is there a critical period for benefit? *Neuroscience* 2006;138:1027-1030.
48. Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, menopause, and the aging brain: How basic neuroscience can inform hormone therapy in women. *J Neurosci* 2006;26:10332-10348.
49. Resnick SM, Henderson VW. Hormone therapy and risk of alzheimer disease: A critical time.[see comment][comment]. *JAMA* 2002;288:2170-2172.
50. Murphy DD, Segal M. Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. *J Neurosci* 1996;16:4059-4068.
51. Zhang QG, Han D, Wang RM, Dong Y, Yang F, Vadlamudi RK, Brann DW. From the cover: C terminus of hsc70-interacting protein (chip)-mediated degradation of hippocampal estrogen receptor-{alpha} and the critical period hypothesis of estrogen neuroprotection. *Proc Natl Acad Sci U S A* 2011;108:E617-624.
52. Morrison JH. A mechanism emerges for the critical period hypothesis for estrogen treatment. *Proc Natl Acad Sci U S A* 2011
53. Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, Miller VM, Naftolin F, Santoro N. Keeps: The kronos early estrogen prevention study. *Climacteric* 2005;8:3-12.
54. Liu RS, Lemieux L, Bell GS, Sisodiya SM, Shorvon SD, Sander JW, Duncan JS. A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging

and difference image analysis. *Neuroimage* 2003;20:22-33.
55. Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. *Nat Neurosci* 2003;6:309-315.
56. Dekaban AS. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Ann Neurol* 1978;4:345-356.
57. Bobinski M, Wegiel J, Tarnawski M, Reisberg B, de Leon MJ, Miller DC, Wisniewski HM. Relationships between regional neuronal loss and neurofibrillary changes in the hippocampal formation and duration and severity of alzheimer disease. *Journal of Neuropathology & Experimental Neurology* 1997;56:414-420.
58. Kantarci K, Jack C.R. J. Quantitative magnetic resonance techniques as surrogate markers in alzheimer's disease. *NeuroRx: The Journal of the American Society for Experimental Neurotherapeutics* 2004;1:196-205.
59. Jagust WJ, Zheng L, Harvey DJ, Mack WJ, Vinters HV, Weiner MW, Ellis WG, Zarow C, Mungas D, Reed BR, Kramer JH, Schuff N, DeCarli C, Chui HC. Neuropathological basis of magnetic resonance images in aging and dementia. *Ann Neurol* 2008;63:72-80.
60. Seliger SL, Longstreth WT, Jr., Katz R, Manolio T, Fried LF, Shlipak M, Stehman-Breen CO, Newman A, Sarnak M, Gillen DL, Bleyer A, Siscovick DS. Cystatin c and subclinical brain infarction. *J Am Soc Nephrol* 2005;16:3721-3727.
61. Schmidt R, Ropele S, Enzinger C, Petrovic K, Smith S, Schmidt H, Matthews PM, Fazekas F. White matter lesion progression, brain atrophy, and cognitive decline: The austrian stroke prevention study. *Ann Neurol* 2005;58:610-616.
62. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med* 2003;348:1215-1222.
63. DeCarli C, Miller BL, Swan GE, Reed T, Wolf PA, Carmelli D. Cerebrovascular and brain morphologic correlates of mild cognitive impairment in the national heart, lung, and blood institute twin study. *Arch Neurol* 2001;58:643-647.
64. Kantarci K, Petersen RC, Przybelski SA, Weigand SD, Shiung MM, Whitwell JL, Negash S, Ivnik RJ, Boeve BF, Knopman DS, Smith GE, Jack CR, Jr. Hippocampal volumes, proton magnetic resonance spectroscopy metabolites, and cerebrovascular disease in mild cognitive impairment subtypes. *Arch Neurol* 2008;65:1621-1628.
65. Luchsinger J, Brickman A, Reitz C, Schupf N, Manly J, Tang M, Small S, Mayeux R, DeCarli C, Brown T. Cerebrovascular disease in mild cognitive impairment. *Alzheimer's & Dementia* 2008;4:T131.
66. Greenberg DL, Payne ME, MacFall JR, Provenzale JM, Steffens DC, Krishnan RR. Differences in brain volumes among males and female hormone-therapy users and nonusers. *Psychiatry Res* 2006;147:127-134.
67. Low LF, Anstey KJ, Maller J, Kumar R, Wen W, Lux O, Salonikas C, Naidoo D, Sachdev P. Hormone replacement therapy, brain volumes and white matter in postmenopausal women aged 60-64 years. *Neuroreport* 2006;17:101-104.
68. Boccardi M, Ghidoni R, Govoni S, Testa C, Benussi L, Bonetti M, Binetti G, Frisoni GB. Effects of hormone therapy on brain morphology of healthy postmenopausal women: A voxel-based morphometry study. *Menopause* 2006;13:584-591.
69. Eberling JL, Wu C, Haan MN, Mungas D, Buonocore M, Jagust WJ. Preliminary evidence that estrogen protects against age-related hippocampal atrophy. *Neurobiol Aging* 2003;24:725-732.
70. Lord C, Buss C, Lupien SJ, Pruessner JC. Hippocampal volumes are larger in postmenopausal women using estrogen therapy compared to past users, never users and men: A possible window of opportunity effect. *Neurobiol Aging* 2008;29:95-101.
71. Erickson KI, Colcombe SJ, Raz N, Korol DL, Scalf P, Webb A, Cohen NJ, McAuley E, Kramer AF. Selective sparing of brain tissue in postmenopausal women receiving hormone replacement therapy. *Neurobiol Aging* 2005;26:1205-1213.
72. Ghidoni R, Boccardi M, Benussi L, Testa C, Villa A, Pievani M, Gigola L, Sabattoli F, Barbiero L, Frisoni GB, Binetti G. Effects of estrogens on cognition and brain morphology:

Involvement of the cerebellum. *Maturitas* 2006;54:222-228.
73. Ha DM, Xu J, Janowsky JS. Preliminary evidence that long-term estrogen use reduces white matter loss in aging. *Neurobiol Aging* 2007;28:1936-1940.
74. Resnick SM, Espeland MA, Jaramillo SA, Hirsch C, Stefanick ML, Murray AM, Ockene J, Davatzikos C. Postmenopausal hormone therapy and regional brain volumes: The whims-mri study. *Neurology* 2009;72:135-142.
75. Coker LH, Hogan PE, Bryan NR, Kuller LH, Margolis KL, Bettermann K, Wallace RB, Lao Z, Freeman R, Stefanick ML, Shumaker SA. Postmenopausal hormone therapy and subclinical cerebrovascular disease: The whims-mri study. *Neurology* 2009;72:125-134.
76. Kuller LH, Margolis KL, Gaussoin SA, Bryan NR, Kerwin D, Limacher M, Wassertheil-Smoller S, Williamson J, Robinson JG. Relationship of hypertension, blood pressure, and blood pressure control with white matter abnormalities in the women's health initiative memory study (whims)-mri trial. *J Clin Hypertens (Greenwich)* 2010;12:203-212.
77. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. Toward defining the preclinical stages of alzheimer's disease: Recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimers Dement* 2011;7:280-292.
78. Kantarci K, Lowe V, Przybelski SA, Weigand SD, Senjem ML, Ivnik RJ, Preboske GM, Roberts R, Geda YE, Boeve BF, Knopman DS, Petersen RC, Jack CR, Jr. Apoe modifies the association between a-beta load and cognition in cognitively normal older adults. *Neurology in press* 2011
79. Bracamonte MP, Miller VM. Vascular effects of estrogens: Arterial protection versus venous thrombotic risk. *TRENDS in Endocrinology and Metabolism* 2001;12:204-209.
80. Bracamonte MP, Rud KS, Owen WG, Miller VM. Ovariectomy increases mitogens and latelet-induced proliferation of arterial smooth muscle. *Am J Physiol Heart Circ Physiol* 2002;283:H853-H860.
81. Lewis DA, Avsar M, LaBreche P, Bracamonte MP, Jayachandran M, Miller VM. Treatment with raloxifene and 17beta-estradiol differentially modulates nitic oxide and prostanoids in venous endothelium and platelets of ovariectomized pigs. *J Cardiovasc Pharmacol* 2006;48:231-238.
82. Jayachandran M, Miller VM. Ovariectomy upregulates expression of estrogen receptors, nos, and hsps in porcine platelets. *Am J Physiol:Heart Circ Physiol* 2002;283:H220- H226.
83. Jayachandran M, Miller VM. Human platelets contain estrogen receptor a, caveolin-1 and estrogen receptor associated proteins. *Platelets* 2003;14:75-81.
84. Jayachandran M, Mukherjee R, Steinkamp T, LaBreche P, Bracamonte MP, Okano H, Owen WG, Miller VM. Differential effects of 17b-estradiol, conjugated equine estrogen and raloxifene on mrna expression, aggregation and secretion in platelets. *Am J Physiol: Heart Circ Physiol* 2005;288:H2355-H2362.
85. Jayachandran M, Owen WG, Miller VM. Effects of ovariectomy on aggregation, secretion, and metalloproteinases in porcine platelets. *Am J Physiol: Heart Circ Physiol* 2003;284:H1679-H1685.
86. Lewis DA, Bracamonte MP, Rud KS, Miller VM. *Genome and hormones: Gender differences in physiology* selected contribution: Effects of sex and ovariectomy on responses to platelets in porcine femoral veins. *J Appl Physiol* 2001;91:2823-2830.
87. Stellos K, Panagiota V, Kogel A, Leyhe T, Gawaz M, Laske C. Predictive value of platelet activation for the rate of cognitive decline in alzheimer's disease patients. *J Cereb Blood Flow Metab* 2010;30:1817-1820.
88. Hochstrasser T, Ehrlich D, Marksteiner J, Sperner-Unterweger B, Humpel C. Matrix metalloproteinase-2 and epidermal growth factor are decreased in platelets of alzheimer patients. *Curr Alzheimer Res* 2011
89. Jablonski M, Maciejewski R, Januszewski S, Ulamek M, Pluta R. One year follow up in

ischemic brain injury and the role of alzheimer factors. *Physiol Res* 2011
90. Jayachandran M, Litwiller RD, Owen WG, Heit JA, Behrenbeck TR, Mulvagh SL, Araoz PA, Budoff MJ, Harman SM, Miller VM. Characterization of blood borne microparticles as markers of premature coronary calcification in newly menopausal women. *Am J Physiol Heart Circ Physiol* 2008;295:931-938.
91. Jayachandran M, Litwiller RD, Owen WG, Miller VM. Circulating microparticles and endogenous estrogen in newly menopausal women. *Climacteric* 2009;12:177-184.
92. Jayachandran M, Litwiller RD, Lahr BD, Bailey KR, Owen WG, Mulvagh SL, Heit JA, Hodis HN, Harman SM, Miller VM. Alterations in platelet function and cell-derived microvesicles in recently menopausal women: Relationship to metabolic syndrome and atherogenic risk. *J Cardiovasc Transl Res* 2011;4:811-822.
93. Berckmans RJ, Neiuwland R, Boing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost* 2001;85:639-646.
94. Morel O, Toti F, Hugel B, Camoin-Jau L, Dignat-George F, Freyssinet JM. Procoagulant microparticles: Disrupting the vascular homeostasis equation? *Arterioscler Thromb Vasc Biol* 2006;26:2594-2604.
95. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. *Cardiovasc Res* 2003;59:277-287.
96. Zwaal RF, Schroit AJ. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood* 1997;89:1121-1132.
97. Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, Hennekens CH, Buring JE. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women.[see comment]. *New England Journal of Medicine* 2005;352:1293-1304.
98. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: A guideline from the american heart association. *Circulation* 2011;123:1243-1262.
99. Rocca WA, Grossardt BR, Shuster LT. Oophorectomy, menopause, estrogen treatment, and cognitive aging: Clinical evidence for a window of opportunity. *Brain Res* 2011;1379:188-198.
100. Price JC, Klunk WE, Lopresti BJ, Lu X, Hoge JA, Ziolko SK, Holt DP, Meltzer CC, DeKosky ST, Mathis CA. Kinetic modeling of amyloid binding in humans using pet imaging and pittsburgh compound-b. *J Cereb Blood Flow Metab* 2005;25:1528-1547.
101. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in spm using a macroscopic anatomical parcellation of the mni mri single-subject brain. *NeuroImage* 2002;15:273-289.
102. Kantarci K, Senjem ML, Lowe VJ, Wiste HJ, Weigand SD, Kemp BJ, Frank AR, Shiung MM, Boeve BF, Knopman DS, Petersen RC, Jack CR, Jr. Effects of age on the glucose metabolic changes in mild cognitive impairment. *AJNR Am J Neuroradiol* 2010;31:1247-1253.
103. Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, Holtzman DM, Zlokovic BV. Apoe isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J Clin Invest* 2008;118:4002-4013.
104. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of alzheimer's amyloid-ss(1-40) peptide from brain by ldl receptor-related protein-1 at the blood-brain barrier. *J Clin*

Invest 2000;106:1489-1499.
105. Morris JC, Roe CM, Xiong C, Fagan AM, Goate AM, Holtzman DM, Mintun MA. Apoe predicts amyloid-beta but not tau alzheimer pathology in cognitively normal aging. *Ann Neurol* 2010;67:122-131.
106. Peskind ER, Li G, Shofer J, Quinn JF, Kaye JA, Clark CM, Farlow MR, DeCarli C, Raskind MA, Schellenberg GD, Lee VM, Galasko DR. Age and apolipoprotein e*4 allele effects on cerebrospinal fluid beta-amyloid 42 in adults with normal cognition. *Arch Neurol* 2006;63:936-939.
107. Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, Ayutyanont N, Keppler J, Reeder SA, Langbaum JB, Alexander GE, Klunk WE, Mathis CA, Price JC, Aizenstein HJ, DeKosky ST, Caselli RJ. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for alzheimer's disease. *Proc Natl Acad Sci U S A* 2009;106:6820-6825.
108. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein e in alzheimer's disease and other neurological disorders. *Lancet Neurol* 2011;10:241-252.
109. Ho AJ, Hua X, Lee S, Leow AD, Yanovsky I, Gutman B, Dinov ID, Lepore N, Stein JL, Toga AW, Jack CR, Jr., Bernstein MA, Reiman EM, Harvey DJ, Kornak J, Schuff N, Alexander GE, Weiner MW, Thompson PM. Comparing 3 t and 1.5 t mri for tracking alzheimer's disease progression with tensor-based morphometry. *Hum Brain Mapp [Epub ahead of print]* 2009
110. Mathis CA, Wang Y, Holt DP, Huang GF, Debnath ML, Klunk WE. Synthesis and evaluation of 11c-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. *J Med Chem* 2003;46:2740-2754.
111. Ono M, Wilson AA, Nobrega J, Westaway D, Verhoeff P, Zhuang Z-P, Kung MP, Kung HF. 11c-labeled stilbene derivative as a-beta aggregate-specific pet imaging agents for alzheimer's disease. *Nuclear Medicine & Biology* 2003;30:565-571.
112. Gunter JL, Shiung MM, Manduca A, Jack CR, Jr. Methodological considerations for measuring rates of brain atrophy. *Journal of Magnetic Resonance Imaging* 2003;18:16-24.
113. Freeborough PA, Fox NC. The boundary shift integral: An accurate and robust measure of cerebral volume changes from registered repeat mri. *IEEE Transactions on Medical Imaging* 1997;16:623-629.
114. Jack CR, Jr., Bentley MD, Twomey CK, Zinsmeister AR. Mr imaging-based volume measurements of the hippocampal formation and anterior temporal lobe: Validation studies. *Radiology* 1990;176:205-209.
115. Jack C, Shiung M, Weigand S, O'Brien P, Gunter J, Boeve B, Knopman D, Smith G, Ivnik R, Tangalos E, Petersen R. Brain atrophy rates predict subsequent clinical conversion in normal elderly and amnestic mci. *Neurology* 2005;65:1227-1231.
116. Jack CR, Jr., O'Brien PC, Rettman DW, Shiung MM, Xu Y, Muthupillai R, Manduca A, Avula R, Erickson BJ. Flair histogram segmentation for measurement of leukoaraiosis volume. *J Magn Reson Imaging* 2001;14:668-676.

14. SUPPLEMENTS/APPENDICES