FEMALE ESTROGEN MENOPAUSE MIND and ENERGY (FEMME): The interaction between diabetes and estradiol on human brain metabolism in postmenopausal women

National Clinical Trial (NCT) Identified Number: NCT03681691

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
2.3.1	Added potential risk of menstrual bleeding.	Some women have experienced blood flow greater than "spotting" and similar to that of a monthly period while wearing the estrogen patch. This is an expected and generally low-risk adverse event that was inadvertently omitted from the initial risks section. This possibility has been added to the "risks" section of the protocol.

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STATEMENT OF COMPLIANCE

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

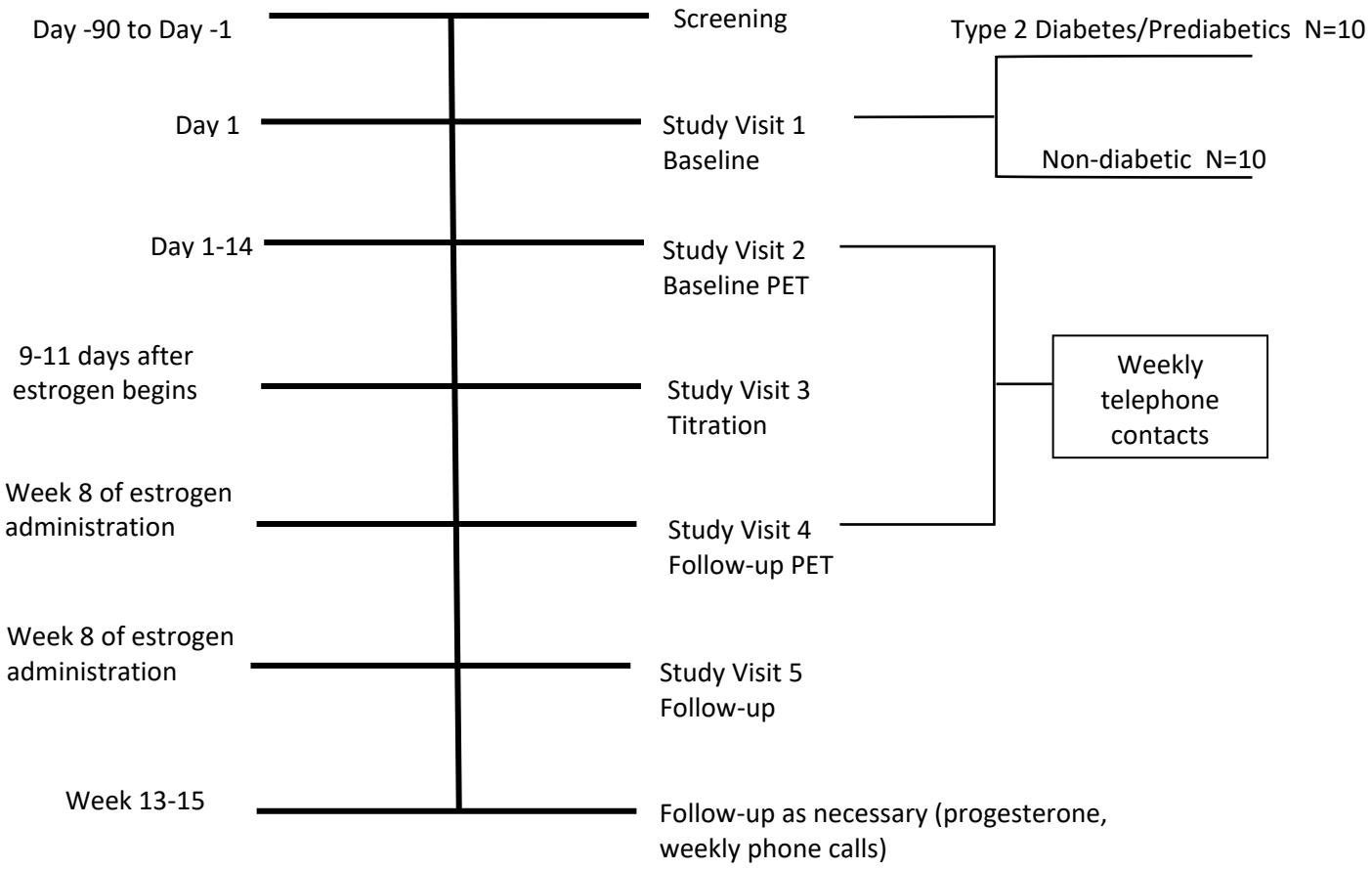
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	The interaction between diabetes and estradiol on human brain metabolism in postmenopausal women
Study Description:	Epidemiological studies suggest there may be an interaction between type 2 diabetes and estrogen in postmenopausal women, such that diabetes may interact with estrogen levels over time to increase risk for dementia. The mechanism for this effect is now known. However, animal research suggests that it may occur through estrogen's effects on cellular metabolism of glucose and ketone bodies. The primary aim of this study is to test whether type 2 diabetes interacts with estradiol on brain metabolism *in vivo* in humans. This will be accomplished by imaging brain metabolism using positron emission tomography before and after short-term administration of transdermal 17β-estradiol in 10 postmenopausal women with diabetes and 10 non-diabetic postmenopausal women.
Objectives:	Primary Objective: The primary objective is to determine whether the effects of glucose and ketone body uptake to the brain in response to 8-week administration of transdermal 17β-estradiol differ in postmenopausal women with and without type 2 diabetes. Secondary Objectives: Secondary objective: 1) Determine whether 8-week administration of transdermal 17β-estradiol alters cognitive performance in postmenopausal women with and without type 2 diabetes.
Endpoints:	Primary Endpoint: Glucose and ketone body uptake in brain measures with positron emission tomography. Regions of interest include the posterior cingulate, temporal cortex, and prefrontal cortex. Secondary Endpoints: Cognitive function assessed using composite scores of short-term memory and executive functioning. The short-term memory composite will be generated by summing z-scores from Story Recall, List Learning, and computerized tasks. The executive function composite will be generated by summing z-scores of Trails part B-A, computerized tasks, and semantic access (letter and category word fluency).
Study Population:	Postmenopausal women aged 60-80 years old will be recruited, 10 with type 2 diabetes and 10 without from the region of Forsyth County, North Carolina.
Phase:	N/A

Description of Sites/Facilities Enrolling Participants:	This is a single-site study that will take place at Wake Forest School of Medicine (WFSM).
Description of Study Intervention:	Participants receive 0.075 mg/day of transdermal 17β-estradiol delivered via a Climara patch for 8 weeks. Estrogen levels will be measured on 9-11 days after estrogen administration begins to titrate levels as needed to attain a circulating level of 50-100pg/ml for each woman. Patches will be changed weekly. The study coordinator will contact participants weekly to monitor safety and ensure compliance. If women have a uterus, progestin will be prescribed at the end of the study to reverse potential endometrial stimulation. All hormone administration will be supervised by Jantira Thomas, DO, Assistant Professor of Internal Medicine, Medical Director Acute Care for the Elderly Unit, Gerontology and Geriatric Medicine.
Study Duration:	It is estimated that the study will last 24-36 months from enrollment to completion of primary data analyses.
Participant Duration:	Participants are expected to take 14-18 weeks (3-4 months) to complete all participant visits.

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening Day -90 to -1	Enrollment/Study Visit 1	Study Visit 2/Day 1-30 Estrogen Day 1*	Estrogen Week 2 SC Phone Call	Study Visit 3/3 Days Post Estrogen Week 2	Estrogen Week 3 SC Phone Call	Estrogen Week 4 SC Phone Call	Estrogen Week 5 SC Phone Call	Estrogen Week 6 SC Phone Call	Estrogen Week 7 SC Phone Call	Study Visit 4 Day 49-56 after Day 1	Study Visit 5 Day 50-56 after Day 1	14-day Progesterone administration (PRN) Day 57 +/-7 days	Final Phone Call Day 71 +/-7 days
Informed consent	X													
Demographics	X													
Medical history	X													
Anthropometrics/Vital signs	X													
Oral Glucose Tolerance Test[1]	X													
Snack	X													
Montreal Cognitive Assessment	X													
Medical Record Review	X													
Medication Review	X													
Fasting blood draw	X	X	X		X[2]						X	X		
Snack		X										X		
Cognitive testing		X										X		
MRI Scan (30 minute)		X										X		
PET Scan			X								X			
Apply Estrogen Patch			X*	X		X	X	X	X	X				
Weekly Phone Calls				X		X	X	X	X	X			X	X
Dispense progestin for daily use													X	

1. Only subjects without diagnosis of diabetes or prediabetes will receive OGTT 2. Non-fasting blood draw to assess serum estradiol levels

2 INTRODUCTION

2.1 STUDY RATIONALE

Type 2 diabetes (T2D) affects an estimated 26% of people over the age of 65[1] and increases their risk for dementia by 50-60%.[2] It also may increase the risk for dementia more in women than in men.[2] One hypothesis suggests that metabolic dysregulation (T2D and insulin resistance) raises the risk of dementia by altering substrate use in bioenergetic pathways to rely less on glucose and more on ketone bodies.[3] Estrogen acts on these same bioenergetic pathways,[4] and evidence is emerging that serum estrogen concentrations may interact with type 2 diabetes to substantially increase the risk of dementia in women. This hypothesis supports the healthy cell bias theory for estrogen action, which hypothesizes that estrogen has beneficial effects in healthy cells, but can have deleterious effects on cells that are affected by diseases such as T2D. The healthy cell bias theory of estrogen action has been demonstrated in animal models, but not yet in humans. Here, we propose to test it in humans by using dual-tracer positron emission tomography (PET) to assess whether estrogen affects the uptake of glucose and ketone bodies differently in women with and without T2D.

2.2 BACKGROUND

Commonly Used Abbreviations

AcAc	Acetoacetate
AD	Alzheimer's Disease
E2	Estradiol
FDG	Fluorodeoxyglucose
HCB	Healthy Cell Bias
MCI	Mild Cognitive Impairment
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
T2D	Type 2 Diabetes
WFSM	Wake Forest School of Medicine

Dementia is a pressing public concern because of its high cost to society and to the individual. Alzheimer's disease (AD) is the most commonly diagnosed form of dementia, estimated to affect 1 in 9 adults over the age of 65 and 1 in 3 over 85. According to the Alzheimer's Association, AD is the 6th leading cause of death in the United States,[5] and a recent publication suggests this may be an under-estimation.[6] Care for AD and other dementias was estimated to cost the US $203 billion in 2013, and is estimated to cost over a trillion dollars by 2050.[5]

AD affects more women than men. For about 2 decades, evidence from large trials has suggested that women may be at greater risk for dementia than men,[7-11] and approximately two-thirds of those diagnosed with AD in the United States are women.[12] As age is a primary risk factor for AD, it has long been thought that the increased risk for dementia in women was due to their longer lifespan.[13] In addition, certain risk factors are more common in women than men, such as lower levels of education and higher risk for depression.[14] However, evidence is emerging that risk factors for AD may affect women differently than men. A population-based study aimed at investigating risk profiles for AD found that risk profiles were different in men and women.[15] Accumulation of misfolded proteins confers greater risk for developing clinical dementia for women than for men; for each unit of AD pathology observed on autopsy, men's risk of clinical dementia increased 3-fold, but women's risk increased 11-fold.[16] In addition, the effect of APOE4, a common risk gene for late-onset AD, was found to confer higher risk for hippocampal atrophy and memory loss in women than in men.[17]

Type 2 diabetes is a potent risk factor for dementia that may differentially affect women. Type 2 diabetes (T2D) affects an estimated 26% of people over the age of 65[1] and increases the risk for dementia by 50-60%.[2] T2D may also increase the risk for dementia more in women than in men.[2] In particular, T2D status may interact with serum levels of estrogen to increase risk for dementia. In a recent study, women with type 2 diabetes who were in the highest quartile of serum estrogen concentrations had 14 times the risk for dementia relative to diabetic women with lower estrogen concentrations.[18] Two recent papers from our group support this finding. Women with type 2 diabetes randomized to receive conjugated equine estrogen therapy in the Women's Health Initiative Memory Study (WHIMS) had an increased risk for dementia and lower brain volumes.[19, 20]

Scientific Premise

One mechanistic explanation for the population-level observation of an interaction between T2D status and estrogen is the healthy cell bias (HCB) theory of estrogen action.[4] The HCB theory posits that the bioenergetic effects of estrogen depend on the metabolic health of the cell they are acting on. Work in animal models shows that estrogen enhances glucose transport and metabolism, mitochondrial respiration, and expression of genes involved in glucose metabolism, and inhibits expression of genes used in ketone body metabolism.[4] Ketone bodies are produced from fat and can be used by cells as an energy substrate. While the brain cannot directly metabolize fat, it can use ketone bodies as a fuel source.[21, 22] The HCB theory posits that in healthy brain cells using glucose as their main substrate, estrogen's glucose-enhancing properties augment the bioenergetic capability of cells; estrogen's action is protective in this situation. However, in cells challenged by stress, aging, or disease, the brain's bioenergetic system may begin to rely on alternate fuel sources such as ketone bodies to meet its heavy

metabolic demands.[4] In this case, estrogen's glucose-enhancing effects may be ineffective. At the same time, estrogen's suppression of ketone use may deprive cells of an alternate energy substrate. Such a scenario could pose a risk to brain health, as cells might not be able to fully meet their energy needs.

Three compounds are generally referred to by the term 'ketone body': acetone, acetoacetate (AcAc) and 3-betahydroxybutyrate (BHB). Acetone is considered to have limited significance in metabolism.[22] The rate of ketone metabolism in the brain is regulated primarily by blood concentration, and secondly by blood brain barrier transport.[22] Ketone bodies enter the brain via diffusion and monocarboxylic acid transporters (MCT1 and MCT2).[22] Elevating circulating ketones, either through infusion or during diabetic ketoacidosis, results in parallel increases in brain metabolism of AcAc and BHB.[23-25] Persistently high levels of ketones, which can occur through prolonged fasting (3.5 days) or a ketogenic diet, increase permeability of the blood-brain barrier to ketones, suggesting an upregulation of transporters.[22, 26] Current evidence suggests that circulating ketones are upregulated in people with T2D.[27-30] This observation supports the idea that ketone bodies may be a viable supplemental bioenergetic substrate in people with T2D, because increased levels of circulating ketones augment availability of ketones to the brain. The premise of this study is to test the HCB theory of estrogen action in humans by measuring brain metabolism of ketone bodies and glucose using a novel dual-tracer PET method before and after administration of estradiol in women with and without diabetes.

Hypothesis

Estrogen affects brain metabolism of ketone bodies and glucose differently in women with T2D and those without, in accordance with the healthy cell bias theory of estrogen action.

Strengths and Weaknesses of Supporting Data

1) Previous results showing interaction between T2D and estrogen or gender on brain and cognitive outcomes are all large trials or epidemiological studies;[2, 18-20] characterization of T2D in these studies lacks relevant details. The WHIMS studies[19, 20] have the advantage that WHIMS is a randomized trial, so the observed effects of estrogen on cognitive and brain imaging outcomes are less likely to be attributable to biases or a confounding factor, as potential confounding factors should be approximately equivalent between the groups. Our study will be an important contribution by examining this potential effect with rigor and detail.

2) The dual-tracer PET has been successfully used to assess difference in ketone body and glucose uptake in healthy younger and older adults[31, 32] and older adults with cognitive impairment.[33] Our preliminary data from an ongoing study (Fig. 2, section 3.4) show the method is sensitive to increased ketone uptake in response to a dietary intervention. Elevated ketone body levels have been observed in people with T2D,[21, 22, 27, 29] but the number of studies is currently small. There is not good representation of women and no research that we know of assessing circulating levels of ketone bodies in people of non-European descent with T2D.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

- **Administration of estrogen**. Estrogen administration has known risks in postmenopausal women. Side effects associated with short–term estrogen replacement therapy may include nausea, breast tenderness, spotting, or bleeding that is sometimes similar to menstrual bleeding

(monthly period). Side effects associated with long-term (several years) estrogen use include the potential for developing thrombosis (blood clot) in the leg, over-growth of the uterine lining that may lead to a type of uterine cancer, and a possible increased risk for breast cancer.

Administration of estrogen in this study will be short term (8 weeks). This dose of estrogen, commonly used in clinical practice, is below that used in previous studies without any medical complications,[34-36] and with a very low incidence of adverse effects (i.e., mild skin irritation at the patch site, slight breast tenderness). If women have a uterus, progestin will be prescribed at the end of the study to reverse potential endometrial stimulation. In addition, estrogen levels will be monitored during the second week of estrogen administration to ensure they are between 50-100pg/ml for each woman. The reference range for circulating levels of estrogen in ovulating premenopausal women used by LabCorp is 85-498 pg/ml. The selected level for this study, 50-100pg/ml, is commonly used in clinical practice because it is within a physiologically active range, but has a low incidence of adverse events. All hormone administration will be supervised by Jantira Thomas, DO (Assistant Professor of Internal Medicine, Medical Director Acute Care for the Elderly Unit, Gerontology and Geriatric Medicine).[37, 38] A study coordinator will contact participants weekly to monitor safety and ensure compliance.

We considered options other than administering estrogen to postmenopausal women to test our hypothesis. Three constraints are important to understand the options we rejected. First, the age range selected for the study (60-80 years) is based on the few studies that have observed this effect in women. The 3 Cities Study reported on women in this age range,[18] and this was also the approximate age range in the Women's Health Initiative Memory Study (WHIMS).[39, 40] We were concerned that changing the age range might alter the outcomes. For a first, proof-of-concept study with a small sample size, it seemed most conservative to specify a tight age range that matched the literature well. Second, the route of estrogen administration and kind of estrogen administered can influence outcomes in the brain. Although human studies are currently limited in number, research suggests transdermal administration of 17β estradiol unopposed by progesterone may be more effective in modifying brain outcomes than other forms of estrogens. [41-45]_ENREF_41 Third, this is a very small, proof-of-concept study that uses a new method to move a theory posited in animal models to a human model. Because the sample size is small, it is important to minimize variability whenever possible.

One option was to bring in postmenopausal women and screen them for naturally occurring estrogen levels in the blood, as was done in the epidemiological 3 Cities Study mentioned above.[18] However, circulating estrogens in postmenopausal women are naturally very low, at the threshold of detection, which could present problems with the reliability of the assays. There was concern on the study team that the findings from the 3 Cities Study might not be replicable by us, as the number of women in the top quartile was quite small, the reported estrogen levels were low, and the findings have not been replicated yet by other groups. In addition, obesity is associated with higher estrogen levels. Indeed, body mass index was higher in the women in the high estrogen quartile in the 3 Cities Study. Stratifying participants based on naturally occurring estrogen levels would likely result in large differences in excess adiposity between high and low estrogen groups. Since obesity may be an independent risk factor for dementia,[46-48] this would introduce a serious confound into the study.

Another option considered was to recruit women who were currently taking hormone replacement therapy and have them cease taking hormones for a period of time. However,

finding enough women in that age range who were taking the appropriate dosage and medication (transdermal 17β estradiol), were willing with the support of their prescribing physicians to stop their medication for the duration of the study, and finding half with type 2 diabetes would likely require screening of a prohibitive number of women. In addition, women who are prescribed estrogen in this age range are not an unbiased sample and may have different characteristics than women who are not prescribed estrogen.

With these considerations in mind, we decided that the best controlled, safest study we could design to test these hypotheses was to bring in a small number of postmenopausal women in the desired age range screened for contraindicated health conditions, and administer transdermal 17β estradiol at a safe, physiological dose for a short period of time with close monitoring and appropriate follow-up.

- ***PET scan.*** Exposure to radiation from PET and paired low-dose CT scans. The amount of whole body radiation dose/participant/year of the Radiation Drug Research Committee (RDRC) is 30 mSv (3 rem). Each woman will be scanned twice and be exposed to a total of 2.62 rem, which is below RDRC limit.
    1. [F-18]FDG PET: 2 x 1.10 rem = 2.2 rem
    2. [C-11]AcAc PET: 2 x 0.0374 rem = 0.0748 rem
    3. Low-dose CT: 4 x 0.084 rem = 0.336 rem

  Participants will be screened for prior radiation exposure within the past year, and be instructed to increase their fluid intake and to void frequently following each PET scanning session to minimize bladder exposure. This radiation dose is not expected to produce any harmful effects, although there is no known minimum level of radiation exposure considered to be totally free of the risk of causing genetic defects or cancer. The risk associated with the amount of radiation exposure participants receive in this study is considered low and comparable to every day risks. Because the targeted population is postmenopausal women, no PET studies will be performed on pregnant or potentially pregnant women or children.

  [C-11] Acetoacetate administration has not demonstrated any toxic effects in patients, as reported by the Cunnane research group at Université de Sherbrooke (143 patients) (Tremblay et al. 2007, *Applied Radiation and Isotopes*, 65(8): 934-940; Nugent et al. 2014, *Neurobiology of Aging*, 35(6): 1386-1395). The radiopharmaceutical preparation has no pharmacological effects. Sodium Acetoacetate C-11 Injection is administered intravenously in 3-6 mL of citrate buffer solution for Injection, USP. The pH of the solution is 3.5-6.5. There is no evidence that administration of [C-11] Acetoacetate increases the risk of ketoacidosis. Based on the maximum amount of Methyllithium reagent used for the synthesis and a 21% reaction yield, the maximum amount of acetoacetic acid synthesized is 2-5 mg. This amount is not significant considering that the amount of acetic acid normally present in the plasma is ~25 mg/L. However, a rare reaction to any of the drugs or procedures to which the participant will be exposed is possible. Thus, a physician will be available at all times during scanning, and an emergency cart will be in close proximity. If adverse effects occur, medical intervention will be provided promptly.

- **MRI scan.** The MRI machine does not use radiation (such as x-rays) and is considered safe. No serious biological effects have been reported from MRI scans. However, operation of the MRI machine produces loud noise. Participants are required to wear earplugs provided at the MRI center. All MRI scans are administered by a trained MRI technician on a research-dedicated

scanner. The technician is trained to help participants insert earplugs and to check whether they have been inserted correctly. With earplugs, the risk to hearing is insignificant.

Some people may experience discomfort in the scanner if they are claustrophobic. All participants will be screened for claustrophobia over the phone and again face-to-face prior to the scan. Participants are given a hand-held device that can be squeezed to signal the MRI technician that they need to stop the scan. If a participant experiences fear of the confined space while in the scanner, they can stop the test at any time.

Metal inclusions in the body are a contraindication for an MRI scan. Participants will be screened over the phone prior to enrollment about potential metal in their bodies. Participants will be screened again face-to-face by study staff the morning of the MRI scan, and then separately screened face-to-face by the trained MRI technician prior to the scan. The MRI suite has MRI-safe gowns, socks, and pants and a private changing area with a locker that locks for participants to store their personal belongings during the scan.

When MRI scans are viewed for research purposes, incidental findings may be observed. In some cases, incidental findings may reveal a previously unknown health concern that would benefit the participant to know about so that they can seek treatment. However, as the scans performed are for research and not diagnostic purposes, incidental findings may not reflect a real problem if a diagnostic scan is performed later. In this case, incidental findings can lead to unnecessary testing. The consent form explains incidental findings in lay language and allows participants to choose if they wish to have incidental findings reported them. Some people do not wish to be given this information. All of our MRI scans will be read by a board-certified neuroradiologist regardless to rule out major abnormalities or issues that might lead to scans being excluded from the study. If an incidental finding is noted and the participant has requested to be notified, we will contact them and provide the feedback from the neuroradiologist. At the participant's request, we will share the research MRI scans with their physician.

- **Blood sampling.** Participants may experience discomfort, bruising and/or bleeding where the needle is inserted. Occasionally, some people become dizzy, lightheaded or feel faint. Infection may occur on rare occasions. To minimize these risks, blood will be drawn only by trained and experienced phlebotomists who will minimize the discomfort as much as possible and will use good clinical practice procedures to reduce risk of infection.

- **Genetic testing.** We will be assessing for presence of the APOE ε4 allele and potentially other cognitively or metabolically relevant genes in the future if new evidence comes to light that these might be important for understanding the results of the study. Genotype information will not be shared with study participants, as is currently recommended research practice for the gene and population we will be studying [49]. Knowing genotype information will not provide any direct benefit to participants, but may cause anxiety. For example, the APOE ε4 allele is established as a risk factor for late onset Alzheimer's disease. However, it is not causal (you may have the allele and never get the disease), there is no known treatment or prevention strategy that targets the presence of the allele, and its predictive ability is diminished after the age of 70 [49]. Therefore, knowing about the risk genotype cannot benefit the participant in terms of treatment, but may needlessly raise anxiety about cognitive decline.

As with other data, genotype information will be identified by subject number only. The Center for Human Genomics will complete the genotyping as for-fee service and will not have access to any identifying information. As only a limited number of cognition-related genetic loci will be characterized, there is not potential for identifying a subject based on genetic information alone as might be a concern for genome-wide studies.

- **Cognitive testing.** Participants will be asked to complete a short battery of cognitive tests. The tests may be given orally, on paper, or on a computer. Participants will be informed that the testing in these visits is not intended or adequate for diagnostic purposes. Careful instructions will be given before the testing. There is a risk that some people may feel frustrated or worried when taking the tests. Participants will be encouraged to communicate with study staff if they are experiencing distress. Study staff will be trained to anticipate this risk and be alert for non-verbal signs of distress. Study staff and participants will both be instructed that testing may be discontinued at any time if the participant chooses.

2.3.2 KNOWN POTENTIAL BENEFITS

There are no anticipated short- or long-term benefits to an 8-week administration of transdermal 17β estradiol in postmenopausal women.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

It is becoming clear that risks for cognitive decline may be different between the sexes. Even if overall risk is the same, it may be that, as in heart disease, different factors contribute risk to development of dementia. Evidence from animal work suggests that the effects of estrogen on bioenergetic pathways may be an important risk factor contributing to lo ng-term declines in glucose uptake in the brain in women, a known precursor to Alzheimer's disease, and a shift in bioenergetic fuel source to more reliance on ketone bodies. This has implications both for administration of estrogen in healthy states, and in common metabolic diseases, such as T2D. However, proof of this theory in humans is entirely absent. A potential mechanism for interaction between common diseases and estrogen is important, because it might lead to changes in the prescribing of estrogens.

The Women's Health Initiative Memory Study (WHIMS) initially published that oral administration of conjugated equine estrogens increased risk for cognitive decline.[50, 51] However, our recent reanalysis of the data taking diabetes status into account showed that the presence of T2D largely accounted for this effect.[19, 20]

While there is no anticipated direct benefit of this study, risks are temporary and reversible. There is a body of literature that examined estrogen therapy as a means to prevent Alzheimer's disease . Multiple studies were published in this age range with this or higher dosage, both transdermal and oral routes, but longer duration of estrogen administration.[34-36, 52-54] Across these studies, side effects were minimal until after 12 weeks of administration, which is 4 weeks longer than the proposed administration here. The study team has planned frequent monitoring to minimize risks further.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary:		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To assess whether 8-week administration of estrogen alters brain glucose and ketone body uptake in women with and without type 2 diabetes.	Brain uptake of glucose and ketone bodies using [F-18]FDG PET and [C-11]AcAc PET.	Using PET tracers allows direct measurement of ketone and glucose uptake to the brain.
Secondary		
To assess whether 8-week administration of estrogen alters cognitive test scores in women with and without type 2 diabetes.	Composite scores of memory and executive function.	The cognitive tests selected are commonly used to assess for dementia and dementia risk.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is an investigation of the effects of transdermal estrogen administration on brain uptake of glucose and ketone bodies in postmenopausal women with and without T2D. The main hypothesis is that estrogen affects brain metabolism of ketone bodies and glucose differently in women with T2D and those without, in accordance with the healthy cell bias theory of estrogen action. All women will be tested before and after 8-week estrogen administration. It is a single-site study with two comparison groups: one with T2D (n=10) and one without (n=10). Randomization is not possible, as the comparison is between women affected and unaffected by T2D (and women cannot be randomized to have T2D). The study will include women of European American and African American descent and with Hispanic ethnicity in proportion to the local population for this age group. No interim analysis is planned.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study will compare the effects of estrogen on brain uptake of glucose and ketone bodies in women with and without T2D. We are comparing women with and without T2D because T2D is a potent risk factor for dementia that may differentially affect women. Type 2 diabetes (T2D) affects an estimated 26% of people over the age of 65[1] and increases the risk for dementia by 50-60%.[2] T2D may also increase the risk for dementia more in women than in men.[2] In particular, T2D status may interact with serum levels of estrogen to increase risk for dementia. In a recent study, women with type 2 diabetes who were in the highest quartile of serum estrogen concentrations had 14 times the risk for dementia relative to diabetic women with lower estrogen concentrations.[18] Two recent papers from our group support this finding. Women with type 2 diabetes randomized to receive conjugated equine estrogen therapy in the Women's Health Initiative Memory Study (WHIMS) had an increased risk for dementia and lower brain volumes.[19, 20]

4.3 JUSTIFICATION FOR DOSE

Women will receive 0.075 mg/day of transdermal estrogen delivered via a Climara patch. Serum estrogen levels will be measured on day 3 of the second week of estrogen administration to titrate levels as needed to attain a circulating level of 50-100pg/ml for each woman. This dosage of estrogen was chosen because it is within physiological limits. Transdermal administration was selected over oral administration because this method of dosing better approximates physiological conditions than oral administration.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Willing to provide written informed consent
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Female, postmenopausal, aged 60-80
4. Normal results on recommended healthcare screenings (e.g., mammogram, pap smear, colonoscopy)
5. BMI 20-35 kg/m^2
6. No evidence of dementia or mild cognitive impairment (MoCA score >25)
7. Women with diabetes, physician diagnosis of type 2 diabetes, or, per American Diabetes Association criteria,[55] showing two markers of dysregulated blood glucose levels at one time (e.g., fasting plasma glucose ≥ 126 mg/dL, 2-hr glucose ≥ 200mg/dL, or elevated HbA1c ≥6.5%) or showing elevated blood glucose using any of these measures at more than one time point.
8. Women with prediabetes, no diagnosis of Type II diabetes and A1c in the pre-diabetic range of 5.7-6.4 or higher.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Use of hormone replacement therapy within the past 3 months
2. History of renal, heart, liver, or neurological disease; head injury with loss of consciousness in the past 5 years; chronic pain, anxiety or depression
3. Presence of medical conditions that might contraindicate estrogen use (e.g., unexplained vaginal bleeding, history of reproductive tissue cancer, thrombosis)
4. Currently taking insulin, metformin, or any other drug or medication judged by the study physician to affect safety or research outcomes of interest
5. Involved in another research study
6. Contraindications for MRI or PET scanning
7. Current smoker
8. Women without diabetes, no physician diagnosis of type 2 diabetes or prediabetes, and evidence of healthy glucose regulation, indicated by an HbA1c <5.7% and fasting plasma glucose <100mg/dL or 2-hr glucose <140mg/dL.
9. Reporting a level of radiation exposure in the past year that would cause radiation levels to exceed recommended limits if the person participates in this study
10. Participants will be excluded for taking metformin or insulin, other hormone replacement therapies, dantrolene (Dantrium, Ryanodex, Revonto), Viekira Pak (ombitasvir-paritaprevir-

Ritonavir-dasabuvir), carfilzomib (Kyprolis), tranexamic acid (Cyklokapron, Lysteda), hemin (Panhematin), lenalidomide (Revlimid), pomalidomide (Pomalyst), or thalidomide (Thalomid).

As this study targets the effects of estrogen on brain post-menopause, men are excluded.

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a current medication, participation in another trial, use of hormone replacement therapy within the past 3 months, availability during study duration, or access to transportation may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

This single-site study aims to recruit 10 postmenopausal women with type 2 diabetes and 10 postmenopausal without type 2 diabetes. All women should be between the ages of 60-80 years and may be of any race or ethnicity. In the Winston-Salem, Forsyth County region, which is the catchment area for this study, approximately 25% of people in this age range identify as minority (primarily African America, less than 1% identify as Hispanic ethnicity). To reflect these demographics, we aim to include 2-3 African American women in each arm of the study.

We anticipate enrolling 1-2 women per month and screening up to 200 women to reach enrollment. Women will be recruited through fliers posted in the general public, hospital, and outpatient clinics; physician outreach in outpatient clinics; advertisement through the VITAL newsletter of the Section on Gerontology and Geriatric Medicine; community talks given by the PI or study staff; and word-of-mouth. Recruitment venues include Wake Forest Baptist Health and Wake Forest School of Medicine clinics, and public venues such as the YMCA, community centers, or churches. Recruitment of historically under-represented populations, such as African American women, will be accomplished through outreach to the African American community through our Aging Center and engagement of historically African American churches in the area. The Aging Center has a strong history of enrolling 25% or more African American participants in research studies.

Study retention will be facilitated by compensating participants for study visits and through weekly contact with the study coordinator to ensure safety and compliance.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Participants will be administered 0.075 mg/day of transdermal estrogen via a Climara patch for 8 weeks.

6.1.2 DOSING AND ADMINISTRATION

The starting dose of estrogen will be 0.075 mg/day administered transdermally with a Climara patch. Serum estrogen levels will be measured 48-96 hours (2-4 days) after administration of the second patch to titrate levels as needed to attain a circulating level of 50-100pg/ml for each woman. Patches will be changed weekly. A higher dose may be administered at the study physician's discretion if the minimum circulating level is less than 50pg/ml. Conversely, the dose may be lowered at the study physician's discretion if the circulating level is greater than 100pg/ml. If advised by the study physician, additional follow up measures of serum estrogen levels may be collected at appropriate time points.

Participants will be instructed to change their patch weekly. A study coordinator will contact them weekly to remind them. If a patch change is missed, the participant will be instructed to change it as soon as possible. If a participant goes 7 days without changing a patch, they will be withdrawn from the study.

Short-term (less than 12 weeks) administration of estrogen has low risk of side effects. Participants will be screened thoroughly to exclude participants with existing risk factors for estrogen treatment. Participants will be contacted weekly by the study coordinator to remind them to change their patch and will be asked a series of questions to screen for potential adverse side effects. As the intervention is being administered as an experimental manipulation, not treatment, if participants experience concerning side effects, they will be encouraged to discontinue use of estrogen, contact their primary care physician as needed, and the study physician will be notified.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Climara patches are a standard treatment, and will be ordered through the pharmacy. The patches will be distributed to participants by study staff. If a participant does not use the patches they are given, they will be requested to return the patches to study staff.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Climara patches are manufactured by Bayer Pharmaceuticals. Each patch comes in an individually sealed protective pouch within a box. Each patch has one adhesive side that is used to adhere to the skin. Per packaging instructions, the adhesive side should be placed on a clean, dry area of the lower abdomen or upper quadrant of the buttock, and should not be applied to or near the breasts. The site selected should not be oily, damaged or irritated, and the waistline should be avoided to prevent tight clothing from rubbing the patch off. Application sites must be rotated, with at least a 1-week interval between applications to the same site. Patches will be labeled as sold.

6.2.3 PRODUCT STORAGE AND STABILITY

It is recommended by the manufacturer that Climara patches be stored between 66-77°F, with excursions in temperature allowed between 59°F and 86°F. The patch should be applied immediately after removal from the protective pouch.

6.2.4 PREPARATION

The Climara patch will require no additional preparation.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

As this is a study to experimentally manipulate estrogen levels in women with and without type 2 diabetes, there is no randomization or blinding.

6.4 STUDY INTERVENTION COMPLIANCE

Compliance will be monitored through serum tests of estradiol levels at baseline, week 2 of estrogen administration, and follow-up. Compliance will also be monitored through weekly phone calls with study staff. If participants miss 7 days of patch wearing, they may be asked to discontinue the study.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements. Participants will be excluded for taking metformin or insulin, other hormone replacement therapies, dantrolene (Dantrium, Ryanodex, Revonto), Viekira Pak (ombitasvir-paritaprevir-Ritonavir-dasabuvir), carfilzomib (Kyprolis), tranexamic acid (Cyklokapron, Lysteda), hemin (Panhematin), lenalidomide (Revlimid), pomalidomide (Pomalyst), or thalidomide (Thalomid).

6.5.1 RESCUE MEDICINE

Participants should take any required rescue medications.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

As this is not a treatment intervention, discontinuation from the Climara patch means discontinuation from the study after an appropriate follow-up for safety. For example, women with an intact uterus should take 14 days of 5 mg/day of progestin to minimize the risk of potential endometrial stimulation, and those who withdraw or are asked to discontinue will be followed appropriately. Remaining study procedures, such as the follow-up blood draws and PET scans should not be completed, as they will not be useful to answer the research question and will expose the participant to risk.

If a participant withdraws from the study, study staff will contact the participant to schedule any necessary follow-up (i.e., administration of progestin) and to collect any unused Climara patches. The same procedure will be followed if the participant is requested to discontinue the study for noncompliance or any other reason.

The data to be collected at the time of study intervention discontinuation will include the reason for withdrawal or discontinuation.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance. Examples of significant non-compliance include missing 7 days of estrogen administration, not communicating with study staff for 3 weeks, and canceling or not showing up for 2 scheduled study visits.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Participant unable to receive the Climara patch for 7 days within the administration period.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Study Completion form. As this intervention is not a treatment trial, any participant who withdraws, drops out, or is discontinued may be replaced if funds and time permit.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if they do not return phone calls from study staff for 3 weeks and/or cancel or do not attend 3 scheduled study visits.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- Study staff will attempt at least twice per week to contact the participant and reschedule the missed visit. If the participant is reached, staff will counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- If study staff phone calls are not returned for at least 1.5 weeks, a letter will be sent to the participant asking them to contact study staff and reminding them of the need for follow-up (if applicable). These contact attempts should be documented in the participant's medical record or study file.
- If study staff phone calls are not returned for 3 weeks, a second letter will be sent informing the participant that we consider them to have dropped out of the study and request that they communicate within the next week so that we can follow-up medically if appropriate.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Lost to follow-up will be minimized by thorough explanation of study requirements during consent, remuneration of the participant for completing study visits, and weekly contact with study staff.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 ASSESSMENTS

The first visit will be a **screening visit (SV)** to determine if participants meet criteria for enrollment. Screening will be completed 0-3 months prior to beginning the experiment and will take approximately 3 hours. The following will take place during screening:

- An IRB-approved written informed consent will be completed prior to any data collection
- Collection of anthropometric information (height, weight, blood pressure)
- Fasting blood draw (complete blood count (CBC), HbA1c, metabolic panel, lipid panel)
- For non-diabetic women, an Oral Glucose Tolerance Test (OGTT) to assess for diabetes status

- Eat a healthy snack
- Demographic and contact information
- Montreal Cognitive Assessment
- Centers for Epidemiological Studies Depression Scale (CESD-10)
- Detailed self-report health history and review of medications, specifically including smoking status, history of hormone replacement therapy, medical conditions that are contraindicated for estrogen use (listed below under chart review), and radiation exposure in the past year.
- Review of medical record to confirm no evidence of potential contraindications to participate. Specifically, chart review will confirm that relevant screenings such as pap smear and mammogram have been completed in compliance with USPTF recommendations and were negative; assess for a history of the following: unexplained vaginal bleeding, breast cancer, ovarian cancer, blood clots, stroke, myocardial infarction, liver disease, kidney disease, hypothyroidism, endometriosis or fibroids, porphyria, lupus, migraines, or tobacco use. Chart review will also be used to confirm the participant is not taking contraindicated medications.

Between 1-30 days after the screening visit, the results of the screening will be reviewed with the study physician to determine eligibility. If the study physician is traveling for an extended period, she may delegate screening review to another physician during that time. The participant will be notified 0-10 days after eligibility is determined and remaining visits will be scheduled as needed.

The second visit will be **study visit 1 (V1)**. V1 will take place 0-3 months after screening. The following will take place during V1, which will last approximately 2 hours:

- Fasting blood draw for future research, serum estrogen, cortisol, glucose, and insulin levels
- Snack
- Cognitive testing. Cognitive testing will include assessment of executive function, working memory, reaction time, episodic memory and verbal memory using a combination of handwritten and computerized tests that can be accomplished in less than 60 minutes.
- 60 minute MRI scan. Participants will have images taken of their brain and may be asked to view images and make a button press in response during the scan.

The third visit will be **study visit 2 (V2)**. V2 will take place preferably 1-14 days after V1 but, depending on participant, equipment and staff availability, may take place up to 30 days after V1 and will last approximately 3 hours. Participants will be required to fast for 6-7 hours prior to undergoing the PET scan. The following will take place during V2:

- Dual tracer PET scan during which time blood draws will take place to assess ketone bodies (betahydroxy butyrate (BHB))

At the conclusion of V2, participants will be given 8 Climara patches. Written instructions for using the patches will be provided and reviewed verbally by study staff. Participants may begin estrogen administration after the PET scan or within 48 hours after completing the PET scan, confirmed by a phone call with the study coordinator. Participants will wear a Climara patch for transdermal estrogen administration for 8 weeks, changing the patch weekly.

Study visit 3 (V3), the fourth visit, will take place 48-96 hours (2-4 days) after administration of the second estrogen patch and will consist of a blood draw (not fasting) to assess serum estradiol levels and genetic assessment [CH1]Assay levels will be returned within 1 week. If levels are less than 50pg/ml or greater than 100pg/ml, they will be provided to the study physician for review. The study physician will

then determine whether the dosage of transdermal estrogen should be increased or decreased to attain the desired range of serum estrogen, and if further blood draws are necessary to assess serum estrogen levels.

Study visits 4 (V4) and 5 (V5) will take place during week 8 of estrogen administration. Participants will be required to fast for 6-7 hours prior to undergoing the PET scan. The following will take place during V4:

- Dual tracer PET scan during which time blood draws will take place to assess ketone bodies (betahydroxy butyrate (BHB))

The following will take place during **V5**:

- Fasting blood draw
- Snack
- Cognitive testing
- 30 minute MRI scan

If the participant has a uterus, estrogen will be continued for 2 additional weeks along with progestin for 10-14 days to reverse potential endometrial stimulation.

Results of blood tests, such as fasting glucose, HbA1c, OGTT, and lipid panel will be provided to participants at their request. If a participant scores lower than a 24 (or 1.5 standard deviations below their age, race, and education adjusted normative score) on the MoCA, when they are contacted to inform them they are not eligible for the study, they will be informed that they may wish to follow-up with their physician for a more detailed cognitive screening. All MRI scans will be viewed by a neuroradiologist. During the consent process, participants will be given the option of having any incidental findings noted by the neuroradiologist reported to them or their physician if they wish, and copies of the scans may be provided by request as well.

DETAILS OF PET SCAN METHODOLOGY AND PROCEDURES

Method of assay and instrumentation. The radioactive drug will be assayed in a Capintec radioisotope calibrator. The capintec in use will be checked for linearity quarterly and constancy daily.

Radioactive drug chosen. [18F]Fluorodeoxyglucose (or FDG) is often used in both research and clinical settings as a surrogate of glucose metabolism. In Alzheimer's disease, use of [18F]FDG is a powerful technique providing a signature pattern of brain regions with impaired glucose metabolism. The metabolic changes seen in Alzheimer's disease are evident even in preclinical stages of the disease [56]. Thus, lending this technique to applications in understanding the impact of brain glucose metabolism and disease progression. [11C]Acetoacetate (or AcAc) is a novel radiotracer with primary applications in study of acetoacetate or ketone body metabolism.

We will use an established method of production for [11C]AcAc that has been used in other institutions, including by our collaborators led by Dr. Stephen Cunnane, PhD, Université de Sherbrooke [32, 33, 57-60]. The production of this radiotracer is published and an automated synthesis and injection unit has been established at WFU PET center. The utilization of both [11C]AcAc and [18F]FDG in an established dual-tracer PET scan may provide valuable insight into impaired brain metabolism, how impaired brain metabolism is linked with cognitive decline, and how it may be modified by interventions.

Pharmacological dose of [11C]AcAc. Consistent with 21 CFR 361.1, there has been no reported pharmacological effect caused by [11C]AcAc (143 patients performed). Quality control specifications included:

- Bubble Point Filter Membrane Integrity
- Visual inspection (clear and colorless solution without presence of particulate matter)
- Radiochemical purity >90%;
- Demonstration of sterility and apyrogenecity.

Quality of radioactive drug. We will synthesize [11C]AcAc as described in our local drug master file (LDMF) and batch record sheets located in the radiopharmaceutical laboratory and attached to this application. Final product [11C]AcAc will have:

- A radiochemical purity of >90%
- Sterility and apyrogenicity testing performed as described in LDMF and will be recorded in batch record files.

[18F]FDG is an FDA approved drug and will be obtained from an FDA approved radiopharmacy.

PET scan procedure.

- Subjects will report to the ground floor of the MRI building to the Molecular Imaging Research PET/CT scanner at Wake Forest Baptist Medical Center.
- Vital signs (blood pressure, heart rate, temperature) and body weight will be within 30 minutes of radiotracer injection.
- An indwelling catheter will be placed into a forearm vein in both arms.
- 30 minutes prior to the PET scan, a heating pad is wrapped around one arm (44 degrees Celcius). Up to 10mL of blood for Glucose, AcAc, and Beta-hydroxybutyrate (or BHB, the other major ketone body) quantification. This blood is stored on ice and immediately processed for analysis after the PET scan.

Part 1 of Scan (0-30 minutes):

- Prior to PET acquisition, a low dose CT scan of the head will be obtained for attenuation correction.
- Patients will be injected with an intravenous bolus of up to 5-10mCi (370 MBq) (+/- 10%) of [11C]AcAc (over 2 minutes). This is the standard dose used in published clinical studies using [11C]AcAc (3-5).
- The PET acquisition will begin immediately after the tracer injection and last for a total of 30 minutes. 2 ml of blood will be sampled from the forearm vein at 3, 6, 8, 12, 20, and 28 minutes post [11C]AcAc tracer injection. This will be used for quantification of radiation estimates throughout the scan-period.

Washout Period (30-60 minutes):

- A washout (“washing”) period will occur from 30-60 minutes after the injection of the first tracer. This will be used to ensure most of the first tracer has been excreted prior to injection of the [18F]FDG tracer and second part of the dual-tracer scan.

Part 2 of Scan (60-120 minutes):

- Prior to PET acquisition, a low dose CT scan of the head will be obtained for attenuation correction.

- Patients will be injected with an intravenous bolus of 5mCi (370 MBq) (+/- 10%) of [18F]FDG (over 20 seconds). This is the standard dose used in published clinical studies using [18F]FDG (3-5).
- The PET acquisition will begin immediately after the tracer injection and last for a total of 60 minutes.
- 2ml of blood will be sampled from the forearm vein at 3, 8, 16, 24, 35, and 55 minutes post [18F]FDG tracer injection. This will be used for quantification of radiation estimates throughout the scan-period.

General Procedures:

- Patients will be positioned in the PET/CT scanner by trained staff. Subjects will be monitored via audio microphone and video. During PET imaging, subjects must remain as still as possible and thus every effort will be made to provide for their comfort including blankets, pillows and diligent assessment. The dual-tracer [11C]AcAC/[18F]FDG PET brain imaging procedure is similar to that used by previous clinical trials utilizing the technique (3-5). Scanning will begin with a standard low dose CT transmission scan of the head that will be used to correctly position the patient and for attenuation correction. For the [11C]AcAc scan brain emission images will be acquired continuously for a 30 minute period. For the [18F]FDG scan brain emission images will be acquired continuously for a 60 minute period. The images will be immediately assessed for technical validity by the PET technologist and if considered inadequate due to motion or other artifact, the subject will have an additional 20 minutes of continuous imaging.
- From the time of first tracer injection until after the whole imaging session is complete, patients will be observed continuously for signs of adverse events or serious adverse events. The injection site will be observed for excessive inflammation or damage to the surrounding tissue.
- A physician or a person designated by the physician with appropriate training and experience will be present during the tracer injection and to approve discharge of the subject from the PET suite.
- Study participants will be contacted 24-72 hours after they are injected with [11C]AcAC/[18F]FDG to inquire if any adverse events occurred within 24 hours of injection.

8.2 SAFETY AND OTHER ASSESSMENTS

The primary safety assessments are assessing medical history through self-report and chart review, and weekly phone calls with participants to monitor potential side effects of estrogen administration. If a participant is found to have a contraindication in their medical history, they will be informed that they are not eligible to participate within 1 month of this determination. If a participant is taking a contraindicated medication, they will also be informed they are not eligible to participate. If the participant wishes to cease taking the medication in order to participate in the study, they will be asked to provide a letter from their physician that this is acceptable, and the risks and consequences of this will be reviewed with the study physician prior to enrolling them.

If a participant reports a concerning side effect, the study physician or a proxy physician will be notified immediately to determine appropriate next steps. If the participant reports vaginal bleeding, this will be reported to the study physician and the participant will be advised to follow up with their primary care provider.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event (AE) is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

The following scale will be used to grade adverse events:

1. **Mild:** no intervention required; no impact on activities of daily living (ADL)
2. **Moderate:** minimal, local, or non-invasive intervention indicated; moderate impact on ADL
3. **Severe:** significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to

concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 EXPECTEDNESS

The PI and/or study physician will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. This study includes administration of transdermal estrogen. According to the package insert, expected risks when administering estrogens include the list below.

Less common but serious:

- Breast cancer
- Cancer of the uterus
- Stroke
- Heart attack
- Blood clots
- Dementia
- Gallbladder disease
- Ovarian cancer

Warning signs of serious side effects:

- Breast lumps
- Unusual vaginal bleeding
- Dizziness and faintness
- Changes in speech
- Severe headaches
- Chest pain
- Shortness of breath
- Pains in your legs
- Changes in vision
- Vomiting

Common side effects:

- Headache
- Breast pain
- Irregular vaginal bleeding or spotting
- Stomach/abdominal cramps, bloating
- Nausea and vomiting
- Hair loss

Other side effects:

- High blood pressure
- Liver problems
- High blood sugar
- Fluid retention
- Enlargement of benign tumors of the uterus ("fibroids")
- Vaginal yeast infection

It is worth noting that these are risks for administration of any estrogens and for long duration. Many of these risks were established from the Women's Health Initiative Study, a landmark study that used long-term oral administration of conjugated equine estrogens. Reported risks from research using short-term administration of transdermal estradiol have been minimal.[34-36, 52, 54]

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, study staff will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Any major AE, i.e., any serious injury including all SAEs that are unanticipated, serious, and possibly related to the study intervention will be recorded and reported to the PI immediately after completing any and all actions that are necessary to protect the subject's health and safety. A description of the event and the date and location of the event will be recorded on the AE Reporting Form. Reported events will be compiled to submit to the IRB and NIA annually, unless it is a serious event that requires more immediate reporting.

- Unexpected fatal or life-threatening AEs related to the intervention will be reported to the IRB and NIA within 3 days of the investigator becoming aware of the event. Other serious and unexpected AEs related to the intervention will be reported within 7 days.
- Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the IRB and NIA on an annual basis.
- All other AEs documented during the course of the trial will be reported to the IRB and NIA on an annual basis by way of inclusion in the annual report.

8.3.6 REPORTING EVENTS TO PARTICIPANTS

During the consenting process, incidental findings will be explained to the participant. Participants will be informed that incidental findings may reveal a previously unknown health concern that it would benefit the participant to know about so that they can seek treatment. However, because the tests we are performing are for research and not diagnostic purposes, incidental findings may not reflect a real problem if a diagnostic scan is performed later. In this case, incidental findings can lead to unnecessary testing.

Participants will be informed of study-related results under the following circumstances.

- If a participant does not qualify for the study at screening, they will be informed why they did not qualify. If they did not qualify because a screening measure was out of range and may indicate an underlying health concern (incidental finding), that will be explained. The nature of incidental findings will also be reviewed with the participant.
- When MRI scans are viewed for research purposes, incidental findings may be observed. In some cases, incidental findings may reveal a previously unknown health concern that it would benefit the participant to know about so that they can seek treatment. However, in our experience, some people do not wish to be informed of MRI-related incidental findings. Therefore, during consent, participants will be asked if they wish to be informed of MRI-related incidental findings. All of our MRI scans will be read by a board-certified neuroradiologist regardless to rule out major abnormalities or issues that might lead to scans being excluded from the study. If an incidental finding is noted and the participant has requested to be notified, we will contact them and provide the feedback from the neuroradiologist. At the participant's request, we will share the research MRI scans with their physician.

8.3.7 REPORTING OF PREGNANCY

All women in the study will be postmenopausal.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The PI and study staff will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB).

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB within 7 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 14 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 31 days of the IRB's receipt of the report of the problem from the investigator.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Aim 1. Assess whether T2D alters cognitive response to 8 weeks of transdermal estrogen. Composite *z*-scores will be calculated by calculating a *z*-score for each cognitive task and summing *z*-scores for the tasks designated as Short-Term Memory (STM Composite) and Executive Function (EF Composite). We will assess differences in follow-up scores between intervention groups using ANCOVA after covariate adjustment for baseline scores using SAS software.[61] Because age, BMI, and education may influence changes in cognition over time (e.g. learning effects), we will assess whether including these as

covariates affects results. The primary outcomes will be the composite scores. Secondary analyses will examine individual components.

Aim 2. Assess whether T2D alters estrogen effects on brain uptake of glucose and ketone bodies in whole brain and Alzheimer's disease (AD) related regions of interest. FDG and AcAc PET scan data will be analyzed with PMOD® Software using methods described by our collaborators.[32] Briefly, imaging data are co-registered to T1 structural MRI data to conduct region of interest (ROI)-based analyses. This will quantify absolute global and regional uptake of FDG and AcAc to determine uptake, as well as uptake of AcAc relative to FDG to find potential areas of compensatory ketone use. T1 MRI images will be processed using publically available software in a customized workflow, primarily ANTs due to evidence of superior performance.[62] Average values for ROI will be calculated for the posterior cingulate/precuneus, temporal lobe, and prefrontal cortices, as these are regions of observed decreases in FDG uptake in AD and are relevant for development and progression of dementia**.** Inference will be based the same approaches as for cognitive data for 1) whole-brain glucose uptake, 2) whole-brain ketone uptake, 3) regional glucose uptake, 3) regional ketone uptake, 4) whole-brain ratio of ketone/glucose uptake, and 5) regional ratio of ketone/glucose uptake. We will assess inter-relationships among changes in these outcomes and with changes in cognitive function using principal components and canonical correlation.

Alternate hypotheses. In addition to its effects on cellular bioenergetics, estrogen can affect vascular health.[63, 64] A recent finding that women with T2D were at increased dementia risk saw increased risk for vascular dementia.[2] Thus, the interaction between T2D and estrogen may be mediated through vascular effects, or through an interaction between metabolic and vascular effects. Therefore, we have included two MRI scans to assess vascular brain health: 1) a T2-weighted FLAIR image to assess white matter hyperintensities, an indicator of small-vessel disease correlated with diabetes status and hypertension,[65-67] and 2) a susceptibility-weighted image to assess for microbleeds.[68]

The 3 City Study[18] found interactions between T2D status and endogenous estrogen levels. The WHIMS study observed an interaction between T2D and women randomized to oral estrogen treatment.[19, 20] We will examine exploratory cross-sectional associations between estrogen levels before estrogen administration and PET and cognitive testing to assess for differences due to endogenous estrogen.

Both the WHIMS and 3 City studies statistically adjusted for BMI. To minimize any potential influence of BMI that may persist after BMI adjustment, we plan to limit participants' BMI range and match for BMI. We will also examine whether changes in BMI or diabetes treatment over follow-up affect results. Cortisol may be elevated in older adults with T2D, even after controlling for BMI[69] and the presence of cortisol may modify the effects of estrogen on cognition.[36] Therefore, serum cortisol will be assayed to test for potential interactions between estrogen and cortisol. Due to the cyclical nature of cortisol secretion, all blood draws will occur between 8AM-10AM. OGTT data will allow us to use glucose and insulin levels as covariates as needed. Drs. Mintz and Hughes are currently collecting pilot data on methods that may enhance the sensitivity of the [^{11}C]AcAc scans. If these methods are successful, they will be incorporated into the final study.

9.2 SAMPLE SIZE DETERMINATION

This is a small, proof-of-concept study to determine whether it is feasible to measure effects of estrogen on brain metabolism using novel PET methods. For ethical (exposure to radiation) and financial reasons, it is not uncommon to limit sample size for PET studies. Previous work specifically examining the effects

of small sample size on PET outcomes observed that in sample sizes comparable to this study, false negatives began to increase, but no increase was seen in false positives. In addition, they concluded good replicability could be achieved in samples of 10-20 PET.[70]

9.3 POPULATIONS FOR ANALYSES

All participants who complete the experimental estrogen administration will be included in the analysis. If a participant discontinues, withdraws, or is lost to follow-up after beginning the study, all efforts will be made to replace that participant and reach the final sample size.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

For all analyses, statistical significance will be defined as $p<0.05$. For all data, distributions will be viewed, assessed for normality, and transformed if needed to conform to the requirements of the selected statistical test.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. The participant will be asked to read and review the IRB-approved consent form. Study staff will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to any procedures being done for the study. A copy of the informed consent document will be given to the participants for their records.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the investigator, the NIA and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and the NIA and will

provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

10.1.3 CONFIDENTIALITY AND PRIVACY

Every effort is made by the investigators, study staff, sponsor(s) and their agents to maintain confidentiality of protected health information collected in the study. All of the materials collected are for research purposes only and will be held in strictest confidence. All WFSM faculty and staff receive annual trainings in privacy, protection of personally identifiable information, and HIPAA regulations. Confidentiality of data is maintained by using research identification numbers that uniquely identify each individual. Data other than demographic information will not use names as an identifier; research ID numbers will be used. Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented.

Written records will be kept in locked cabinets in the Sticht Center on Aging. Files matching participants' names and demographic information with the research ID numbers are kept in a separate room and locked file with a different key from for all other files. Files may be obtained from the research unit only by authorized study personnel. After the study is completed, local data will be stored with other completed research studies in a secured storage area.

Digital records, including brain imaging data, will be stored on password protected, network drives behind the WFSM firewall. These drives are routinely backed up and access to the drives is limited to necessary personnel. Brain imaging data are stored using subject number and date of acquisition as identifiers. The study PI regulates access to the drives used for brain imaging data. Data other than brain imaging data will be entered into REDCap, which allows the PI to control who has access to viewing and downloading data.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants,

Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at Wake Forest School of Medicine. With the participant's approval and as approved by the Institutional Review Boards (IRB), de-identified biological samples may be stored at Wake Forest School of Medicine. These samples could be used for future related research with the permission of the PI. During the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed. When the study is completed, access to study data and/or samples will be provided through permission from the PI and IRB.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

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10.1.6 SAFETY OVERSIGHT

Safety oversight will be monitored by the PI and the study physician, as well as the study team. Any safety concerns should be reported immediately to the PI and/or study physician or proxy for the study physician. Safety concerns will also be addressed at monthly study team meetings. AEs will be reported as described above.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control metrics for non-imaging data will be generated from computerized reports generated from the database after data entry. Monthly reports will identify missing data, values that are out of range, and track attendance, as well as show plots of primary outcome measures to identify outliers that

may signify data entry errors. These metrics can be generated by REDCap. A study staff member will be identified who will routinely generate these measures in REDCap approximately 24 hours prior to the monthly study meeting. The study staff member will present the data to the study team and data errors will be agreed upon by the team. During the meeting, a study staff member will be assigned an action to correct the data entry error. This will be documented in the meeting minutes as an action item and follow-up will be added to the next month's agenda. When data are corrected in REDCap, REDCap software automatically tracks the date, time, and user who modified the database.

At the end of the study, histograms of the data distribution for each variable will be generated to identify outliers that may signify errors in acquisition or data entry. Many variables in the study will be acquired at 2 or 3 time points. For these variables, histograms of differences will be generated. This additional step can identify data acquisition or entry errors through unusual amounts of change.

Quality assessment will performed on MRI and PET data at 3 time points on all data sets and results will be recorded in a REDCap form accessible by all study team members. First, raw data will be viewed after being transferred from the scanner and converted into nifti format to ensure completeness and assess for imaging ghosting and reconstruction errors. After preprocessing, data will be viewed again to assess for errors and residual motion. Finally, data will be viewed to assess any errors during processing. All image processing steps will be verified before summary metrics are extracted for the final statistical analyses. All data quality questions will be referred to the PI.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

As this is a single-site study, all data will be collected by study staff at this site. The header of all data collection forms includes a unique numerical study identifier (study ID number), a unique alphabetical identifier ("acrostic"), the date the form was collected, the initials of the study staff who completed the form, the study name, and the study visit (e.g., baseline visit 1). The footer for all data collection forms includes version number and page.

Types of data collected include 1) paper forms, 2) electronic data, and 3) neuroimaging data. All data collection will be performed by trained study staff. Every effort is made by the investigators, study staff, sponsor(s) and their agents to maintain confidentiality of protected health information collected in the study. All of the materials collected are for research purposes only and will be held in strictest confidence. All WFSM faculty and staff receive annual trainings in privacy, protection of personally identifiable information, and HIPAA regulations.

Written records will be kept in locked cabinets in the Sticht Center on Healthy Aging and Alzheimer's Prevention. Files matching participants' names and demographic information with the research ID numbers are kept in a separate room and locked file with a different key from for all other files. Files may be obtained from the research unit only by authorized study personnel. After the study is completed, local data will be stored with other completed research studies in a secured storage area. After data collection on paper forms, data will be entered into a REDCap database that is password protected and accessible only by study staff in 0-14 days after data are collected. Only the PI will have permission to download identifying information from the REDCap database and the PI will assign data access for all study staff.

Digital records, including brain imaging data, will be stored on password protected, network drives behind the WFSM firewall. These drives are routinely backed up and access to the drives is limited to necessary personnel. Brain imaging data are stored using subject number and date of acquisition as identifiers. The study PI regulates access to the drives used for brain imaging data.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the study protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. All deviations must be addressed in study source documents and must be sent to the reviewing Institutional Review Board (IRB) per their policies.

A computerized tracking system will be used to document participant attendance, generate quality control reports for identifying protocol deviations and to assess protocol adherence. Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate. The study team will generate Study Reports for the DSMB and will provide information on the overall status and timeline of the study including enrollment status, recruitment, subject status (i.e., number completed), safety events, and protocol deviations. Study Report tables will be generated only from aggregate (not by group assignment) baseline and safety data for the study population. Study team meetings will occur at least twice a month, and quality control metrics will be reviewed once a month.

10.1.10 PUBLICATION AND DATA SHARING POLICY

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COC	Certificate of Confidentiality
CRF	Case Report Form
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
MOP	Manual of Procedures
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PET	Positron Emission Tomography
PI	Principal Investigator
QA	Quality Assurance
SAE	Serious Adverse Event
UP	Unanticipated Problem

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Changes	Brief Rationale
2.0	03/13/19	Section 2.31 Add language regarding Genetic testing	Genetic testing was not included in the original IRB approved protocol
2.0	03/13/19	Section 5.1 Inclusion Criteria: remove number 7 regarding transportation.	Transportation is available if necessary.
2.0	03/13/19	Section 5.2 Add exclusionary medications to List of Exclusions	Exclusionary medications were outlined in original protocol under concomitant medications but not in the Exclusion List.
2.0	03/13/19	Section 8.1 Assessments: Add "For non-diabetic women" to bullet regarding OGTT	OGGT is unnecessary for participants with a diagnosis of diabetes.
2.0	03/13/19	Section 8.1 Add language to V2 and V4 to indicate the participants must be fasting for the PET scans	The PET scan requires the participants to fast for 6-7 hours prior to the scan.
3.0	05/06/19	Section 1.3 Add fasting blood draw to V2 and V4 since blood is being drawn at various points of PET scan	The PET scan includes fasting blood draws that were not identified on the Schedule of Assessments
3.0	05/06/19	Section 8.1 Remove fasting blood draw for genetic assessment ad to assess ketone bodies from SV	The blood draw for genetic assessment is done at V3 and the blood draws to assess for ketone bodies are done during the PET Scan at V2 and V4, not at the SV
3.0	05/06/19	Section 8.1 Add fasting blood draw to assess for ketone bodies to V2	This was erroneously included in the SV in the previous protocols.
3.0	05/06/19	Section 8.1 Add blood draw for genetic assessment to V3	This was erroneously included in the SV in the previous protocols.
3.0	05/06/19	Section 8.1 Add fasting blood draw to assess for ketone bodies to V4	This was erroneously included in the SV in the previous protocols.
4.0	09/05/19	Section 1.1 Change study population age range to 60-80	Lowering the inclusion age to 60 should allow for a greater number of participants to qualify
4.0	09/05/19	Section 5.1 Expand BMI range up to 35	Increasing the BMI range should allow a greater number of participants to qualify
4.0	09/05/19	Section 5.1 Change Inclusion Criteria age range to 60-80	Lowering the inclusion age to 60 should allow for a greater

			number of participants to qualify
5.0	**12/09/19**	**5.1 Add Inclusion Criteria #8 to now include prediabetic women to participate**	**Prediabetic women will now be eligible to participate where, in the past, were excluded.**
5.0	**12/09/19**	**5.2 Modify wording of Exclusion Criteria #8 to clarify necessary ranges for cutoff measurements for prediabetic women.**	**Prediabetic women were all excluded in the past and now are included if measurements fall into this specific range.**
6.0	**01/28/2020**	**1.3 Expand length of time between SV1 and SV2 to 1-30 days.**	**Increasing the span of time allows for more flexibility for scheduling basesd on participant, equipment and staff availability.**
6.0	**01/28/2020**	**8.1 Expand length of time between SV1 and SV2 to 1-30 days**	**Increasing the span of time allows for more flexibility for scheduling basesd on participant, equipment and staff availability.**
7.0	**08/14/2020**	**10.1.9 Remove reference to DSMB.**	**There is no Data Safety Monitoring Board for this study.**

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