THE INTERACTION BETWEEN DIABETES AND ESTRADIOL IN POSTMENOPAUSAL WOMEN: CURRENT REPORTS FROM THE FEMALE ESTROGEN MENOPAUSE MIND AND ENERGY (FEMME) STUDY

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LIST OF ABBREVIATIONS

AcAc/FDG: 11C-Acetoacetate/18F-Fluorodeoxyglucose

AD: Alzheimer's Disease

ADRD: Alzheimer's Disease and Related Dementias

AGE: Advanced Glycation End-Products

APOE: Apolipoprotein E

Aβ: Amyloid Beta

BBB: Blood-Brain Barrier

BMI: Body Mass Index

BVRT: Benton Visual Retention Test

CA1: Cornu Ammonis 1

CEE: Conjugated Equine Estrogen

CHD: Coronary Heart Disease

COVID-19: Coronavirus Disease 2019

COX: Cytochrime c Oxidase

CVLT: California Verbal Learning Test

DHEA: Dehydroepiandrosterone

E1: Estrone

E2: Estradiol

E3: Estriol

ELITE: Early versus Late Intervention Trial of Estradiol

EMS: Estrogen Memory Study

ER: Estrogen Receptor

FDG: 18F-Fluoro-Deoxyglucose

FEMME: Female Estrogen Menopause Mind and Energy Study

FMP: Final Menstrual Period

fMRI: Functional Magnetic Resonance Imaging

FNAME: Face-Name Associative Memory Exam

FSH: Follicle-Stimulating Hormone

HbA1c: Glycated Hemoglobin

HCB: Healthy Cell Bias

HDL: High Density Lipoprotein

HERS: Heart and Estrogen/Progestin Replacement Study

HT: Hormone Therapy

IR: Insulin Resistance

KEEPS: Kronos Early Estrogen Prevention Study

KEEPS-COG: Kronos Early Estrogen Prevention Study of Cognitive and Affective Trial

LDL: Low Density Lipoprotein

LH: Luteinizing Hormone

MCI: Mild Cognitive Impairment

MoCA: Montreal Cognitive Assessment

MPA: Medroxyprogesterone Acetate

MRI: Magnetic Resonance Imaging

NODDI-DTI: Neurite Orientation Dispersion and Density Imaging-Diffusion Tensor Imaging

OVX: Ovariectomized

P: Progesterone

PET: Positron Emission Tomography

PFC: Prefrontal Cortex

RAGE: Receptor of Advanced Glycation End-Products

RCT: Randomized Clinical Trial

SWAN: Study of Women's Health Across the Nation

T2D: Type 2 Diabetes

TCA: Tricarboxylic Acid Cycle

WHI-HT: Women's Health Initiative Hormone Therapy

WHIMS: Women's Health Initiative Memory Study

WHIMSY: Women's Health Initiative Memory Study in Younger Women

WHISCA: Women's Health Initiative Study of Cognitive Aging

ABSTRACT

Aging and diabetes are both significant risk factors for Alzheimer's disease (AD) dementia that independently affect brain function and cognition. However, among older women, the interaction between hormone therapy (HT) and these known risk factors is not yet fully understood.

Basic neuroscience studies and observational data support evidence of the protective role of HT, especially estradiol (E2), against cognitive decline and AD dementia. Yet, the large, well-designed Women's Health Initiative Memory Study (WHIMS) suggests that first-time initiation or re-initiation of postmenopausal HT increases the risk for probable dementia in women age 65 years and older. Mechanisms underlying these potential harmful effects of HT on the brain are unknown. Accumulating research reveals that estrogens exert their neuroprotective effect against dementia in people with diabetes differently than those without diabetes. Understanding the effect of E2 on cognitive functioning may help elucidate pathways to dementia in people with diabetes.

This thesis project specifically focuses on an E2-sensitive associative memory-related paradigm using functional magnetic resonance imaging (fMRI) techniques in older women with and without diabetes. As a result, this work explores existing literature from randomized trials, epidemiology, and animal model systems to provide a fundamental understanding into estrogens actions on cognitive and functional changes in aging.

Chapter 1

INTRODUCTION

Emerging evidence suggests that initiating or re-initiating postmenopausal hormone therapy (HT) to women with type 2 diabetes (T2D) may markedly increase their risk for dementia and cognitive impairment in old age[1,2,3]. Diabetes and aging are both significant risk factors for dementia that independently affect brain function and cognition. However, how HT, more specifically estradiol (E2), may interact with these known risk factors remains unclear.

Previous studies on the use of estrogen therapy on cognition provided some insights for E2's actions, being protective/beneficial. Results from the earlier randomized clinical trials (RCT) and observational studies have been both influential and controversial on postmenopausal HT and the benefit of estrogens. Some evidence demonstrated promising benefits for E2 on the expression of the apolipoprotein E (APOE) gene to protect the brain from Alzheimer's disease (AD)[4,5]. However, initial findings from the Women's Health Initiative Memory Study (WHIMS) indicated an increased risk for probable dementia and mild cognitive impairment (MCI) in postmenopausal women (aged ≥65) who initiated or re-initiated conjugated equine estrogen (CEE) HT with or without medroxyprogesterone acetate (MPA)[6,7,8,9,10,11]. WHIMS data raised important questions on the relationship between estrogens and brain aging in older women[11].

The Three-City Study, a population-based prospective study, investigated the association between endogenous E2 and incidence of all-cause dementia in postmenopausal women aged 65 years or older[3]. Among older women who were not currently using HT, those with relatively high E2 levels who had T2D had a 14-fold

increased risk for dementia compared to those with low levels of E2[3]. Interestingly, this observation was strengthened by follow-up analyses of the WHIMS data[1,2]. Post hoc analyses of WHIMS data by Espeland et al. (2015) demonstrated that harmful effects of HT on the brain were driven primarily by women with diabetes. Altogether, these findings indicate that metabolic diseases such as T2D may be a key modifier influencing the effects of HT/E2 in older women.

The aim of this first chapter is to provide a literature review of mechanisms by which E2 and T2D interactively affect brain metabolism and cognitive functioning that may explain higher AD risk in women.

Type 2 Diabetes Increases Risk for Dementia

Type 2 diabetes (T2D) is a metabolic disorder that elevates the risk for dementia by 50-60% and may also influence more women than men[12]. Over 38 million Americans aged 65 years and older are living with prediabetes or T2D[13]. Those with prediabetes are susceptible to develop T2D as they age. Among 5.6 million older adults with Alzheimer's disease and related dementias (ADRD), 37% of them have T2D as a coexisting condition, and two-thirds are postmenopausal women[14].

ADRD and T2D are associated comorbidities that may impact progression and severity of cognitive decline and neurodegeneration in older women. Research supports the associations between T2D and high incidence of neurodegeneration and cognitive impairment seen in AD[15]. T2D is characterized by elevated blood glucose levels, and impaired insulin production and release. Risk factors such as obesity, hyperglycemia and brain insulin resistance (IR) associated with T2D contribute to neuronal death by enabling oxidative stress in neurons[16]. In obesity, adipocytes found in visceral fat, and macrophages

secrete proinflammatory cytokines (IL-1β: interleukin 1 beta; IL-6: interleukin 6; TNF- α: tumor necrosis factor alpha) across the blood-brain barrier (BBB). These inflammatory mediators can induce the activation of stress kinases in neurons, thus leading to insulin signaling impairment, which contributes to neuronal death[16]. In addition, amyloid beta (Aβ) oligomers stimulate the stress kinases and induce microglia activation, which produces more proinflammatory cytokines. As a result, Aβ oligomers trigger oxidative stress, thus aggravating brain IR which contributes to neurodegenerative processes[16]. Impaired insulin signaling may result in tau hyper-phosphorylation implicated in cognitive impairment[15]. In addition to impaired insulin activity, chronic hyperglycemia increases the accumulation of advanced glycation end-products (AGE), which mediate the intra-neuronal transport of circulating Aβ proteins (hallmark of AD) into the brain through the BBB. AGEs also cause mitochondrial dysfunction and oxidative stress. Oxidative stress pathways lead to defects in brain vasculature and metabolism, which promote AD neuropathology[16].

In contrast to hyperglycemia, hypoglycemia and significant reduction in HbA1c (glycated hemoglobin) independent of hypoglycemia may play an important factor in higher dementia incidence due to the adverse effects of low blood sugar levels in older adults with diabetes[16,17]. These should be considered as risk factors for cognitive impairment and dementia in T2D, affecting the progression of cognitive decline. Altogether, functional and neural outcomes of T2D manifest changes in brain vasculature, inflammation and metabolism that are involved in ADRD pathologies. These are implicated in metabolic syndrome, a cluster of risk factors for cardiovascular diseases and diabetes, that may impact the risk of ADRD.

As a result, T2D alters functional connectivity (FC), which may contribute to cognitive deficits seen in AD[18,19]. The impairment of insulin signaling associated with hyperglycemia and brain IR is linked to altered functional connectivity and plays a consequent role in T2D-mediated cognitive impairment and energy metabolism networks. In AD, changes in FC patterns are mostly pronounced in hippocampus, prefrontal cortex, precuneus, and cingulate cortex[20,21,22]. These changes in AD-like regions may be associated with neurodegeneration and cognitive impairment.

Nevertheless, these prior studies did not focus on the sex-specific mechanisms of T2D that may increase ADRD risk in women, leaving much unknown. In T2D, sex-related factors such as prior or current HT and reproductive history may elucidate potential mechanisms that drive a higher risk for ADRD. Recently, investigations into the metabolic effects of HT with diabetes generated in-depth questions toward the increased ADRD risk in aging women[23]. For example, a recent study examined the influence of IR on hippocampal volume and cognitive performance in non-diabetic postmenopausal women (mean age 57.4 years) using HT[24]. This study suggests that IR is associated with decreased hippocampal volumes and worse cognitive performance in middle-aged, postmenopausal HT-user women. Follow-up evidence is needed to evaluate the effect of IR on brain structure and cognitive performance in non-HT user women and also in older women (age >65) with HT and T2D. HT may or may not exacerbate T2D's metabolic effects on neurodegenerative pathologies.

Estrogens in Women

Estrogens are one of the steroids known as "sex hormones" for their roles in reproductive physiology and behavior. Compared to men, women have naturally higher

levels of estrogens[25]. Cumulative exposure to estrogenic stimulation throughout women's reproductive life exerts long-lasting effects in the brain[26]. A recent study using aged, ovariectomized (OVX), E2-treated, female rhesus monkeys observed beneficial effects of E2 on cognition that persisted longer than 2 years after discontinuation of E2-treatment[27]. This suggests that long-lasting effects of estrogens on cognitive performance after cessation of ovarian function can inform about the timing of HT in postmenopausal women. Yet, more evidence is needed to understand the role of HT on the brain in women after the reproductive stage ends[27].

Menopause is simply described as a transition into a non-reproductive stage resulting from loss of ovarian estrogens, mainly 17β-estradiol (E2). There are three menopausal stages before and after the final menstrual period (FMP): pre- (regular menstrual cycles), peri- (menopausal transition with at least one menstrual cycle prior to FMP) and post-menopause (no menstrual cycles after FMP). In pre-menopause and early peri-menopause, the mean integrated E2 level is around 80 pg/ml in a normal 28-day cycle[28,29]. As women transition from pre-menopause to post-menopause, their mean serum E2 levels fluctuate and eventually decrease. To this date, there are no validated E2 cut-points that predict the length of peri-menopause or FMP into post-menopause. In a population-based study, serum E2 levels declined from 64.5 pg/ml (SE=3.6) two years before FMP (peri-menopause) to 21 pg/ml (SE=1.2) two years after FMP (post-menopause)[30].

For decades, research regarding the role of E2 has focused on women's reproductive system and physiology only. Recent evidence indicates that estrogens also

influence non-reproductive behavior including brain, locomotor, cardiovascular and metabolic health[31].

Estrogens and progesterone are the main ovarian hormones and are produced primarily in women's ovaries[32,33]. During the pre-menopausal period, the main circulating form of estrogens is E2. Other types of estrogens include estrone (E1) and estriol (E3). E1 and E2 are the two major biologically active estrogens in non-pregnant women. E3 is the main pregnancy estrogen with no roles in non-pregnant premenopausal women and men. E2 can be oxidized reversibly to E1, and both E2 and E1 can be converted to E3. E3, from the conversion of E1, plays a large role in pregnancy when it is produced in large quantities by the placenta[34]. In post-menopausal stages, E1 is the main estrogen synthesized in adipose tissue from adrenal dehydroepiandrosterone (DHEA).

The ovary requires both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) released from hypothalamus-pituitary gland of the brain to produce estrogens (E1, E2 and E3). LH stimulates the cells surrounding the follicle to produce progesterone and androgens. Production of progesterone (P) is in the corpus luteum of the ovary and regulates the condition of the inner lining (endometrium) of the uterus. P is also produced by placenta and adrenal glands. In the ovaries, estrogen synthesis begins in theca cells (endocrine cells) with androgen synthesis and ends with conversion of androgens to estrogens in granulosa cells by aromatase, a key enzyme for E2 synthesis. E2 conversion and secretion amounts vary during menstrual cycle, pregnancy and menopause.

In addition to the primary production in the ovary, E2 can be synthesized in non-reproductive (extra-gonadal) tissue and organs such as adipose tissue, liver, kidney, bone, muscles, heart and brain[35]. Moreover, the chemical structure and biological properties of

gonadal and extra-gonadal estrogens are the same, except for the effects and actions of extra-gonadal estrogens, which are more localized. They act and metabolize locally, limiting their systemic effects. This may explain the role of brain-derived E2 in memory and synaptic plasticity.

Adipose tissues are the second major source of circulating estrogens after the gonads. Contribution to estrogens in adipose tissues increases during pre-menopause. E2 synthesis in adipose tissues stimulates the production of high density lipoprotein (HDL) cholesterol and triglycerides. It also controls the levels of production of low density lipoprotein (LDL) and fat deposition. Research shows evidence on E2's beneficial contribution to lipogenesis[35]. Decreases in E2 synthesis in the adipose tissues due to age and menopause may lead to obesity[36]. This explains the weight gain and the increased risk for metabolic syndrome seen in T2D in postmenopausal women[37,38]. Metabolic syndrome contributes to IR and impaired brain vasculature over time to reduce brain health.

Emerging evidence indicates that the human brain can synthesize E2 and P locally[39,40,41,42,43]. However, the brain is mainly affected by ovarian E2. E2 modulates memory-related synaptic plasticity in the hippocampus and could potentially improve memory circuitry. It also regulates gene expression, neuronal survival, neuronal and glial differentiation and synaptic transmission acting on estrogen receptors (ER)[42,43]. The main sites of E2 actions in the brain are prefrontal cortex (PFC) and hippocampus, which are involved in learning (encoding) and memory (storage) processes. Additionally, ovarian hormones play regulatory roles in thalamus, hypothalamus, pituitary gland, cerebellum and brain stem[40]. Both ER-α and ER-β are abundant in the hippocampus and PFC and are involved in neuronal migration, axonal guidance, neurogenesis, synaptogenesis and

apoptosis[35]. E2's action through these ERs mediates hippocampal memory processes, cell signaling and synaptic density[44,45]. Therefore, these estrogenic actions via the ER system are crucial for women's brain health, as women may experience accelerated changes in memory and executive functioning following natural (transitional) or surgical (induced) menopause[36].

Evidence in mouse models demonstrate E2's neuroprotective action through the ER system in the brain for anti-apoptotic effects, protection from free radicals, anti-inflammatory effects, regulation of calcium channels and protection via increasing cerebral blood flow[46]. However, menopause and age-related shift in ERα/ERβ expression may reduce neuroprotection, transcription factors, and cell signaling and metabolism in the brain[44,47]. Basic studies and observational research provided support for E2 replacement/HT to mitigate and delay cognitive decline and improve memory performance[47]. E2 reduction in peri-menopause is commonly associated with obesity, sleep disturbance, depression, metabolic syndrome and changes in cognitive function in women[38,48]. Both aging and menopause may impact varying expression of ER-α/ER-β, especially in the hippocampus, to negatively affect attention and cognition[49,50]. This indicates the importance of E2 effects on the brain in women.

Estrogen Actions in the Brain

Estrogen actions via the ER system mediate cognitive processing and function throughout the brain. E2's neurotrophic effects lead to changes in the morphology and electrophysiology of neurons. In animal models using mice and rats, the hippocampus has been shown to play a key role for improvements of learning and memory in response to E2 treatment[51,52,53]. E2's memory-enhancing actions influence hippocampus through

substantial changes in neuron morphology and neurotransmission, including increases in dendritic spines[54]. E2 regulates synapse formation in the cornu ammonis subregion 1 (CA1) of the hippocampus which indicates new connections or strengthening of existing connections[51].

Growing evidence from imaging studies has identified brain activation/deactivation patterns that are sensitive to E2 levels and menopausal stages[55,56,57,58,59]. Changes in E2 levels with age lead to decreases in brain glucose metabolism in frontal, temporal and parietal regions. This indicates that E2 has clear metabolic effects especially in the hippocampus and PFC. These areas are involved in learning and memory circuitry.

Research shows that verbal memory and attention/working memory are the cognitive domains that are most-affected by changes in estrogen levels in women. Some evidence suggests that estrogen therapy may protect against age-related cognitive decline and lower the risk for dementia by exerting its protective effects in estrogen-sensitive brain regions[4,60]. However, growing literature on potential benefits of HT in women shows contradictory results.

To date, how estrogens exert beneficial effects on brain structure, function and metabolism in postmenopausal women remains unclear. While basic and observational studies and smaller RCTs continue to show some cognitive benefits for HT, large, well-designed clinical trials consistently fail to demonstrate such cognitive benefit. Behavioral studies in rats confirm that changes in E2 levels lead to altered performance on memory tasks resulting in bidirectional effects (enhancement vs. impairment) through the ER system in the brain[47,61]. These results suggest that the response to E2 therapy may reflect the cognitive health in postmenopausal stage[62]. In addition, E2 actions may depend on the

cell condition and disease status[63]. The harmful effects of E2 regarding brain structure and function may be due to cells progressing from a healthy stage to unhealthy or being compromised by disease such as T2D. Mounting evidence on HT on the brain, and ADRD risk in women informs researchers on the fundamental roles of E2.

Postmenopausal Hormone Therapy on Cognition and ADRD Risk in Women

Estrogens influence women's reproductive behavior and physiology and also regulate non-reproductive systems including brain and metabolic health. In the early 1990s, conjugated estrogen use for aiding common menopausal symptoms (hot flashes, night sweats and weight gain) significantly increased. Some of these increases in estrogen use occurred among older postmenopausal women, not for treating menopausal symptoms, but based on expectations that it may promote bone, cardiovascular, and brain health. Premarin (conjugated estrogen tablets) was the most-prescribed drug for women, with sales reaching $1 billion in 1992[64].

Early preclinical and clinical research suggested that E2-deficiency in postmenopausal women was associated with changes in learning and memory and the risk of developing dementia[47,65,66,67] . Evidence showed that replacement of E2 was a feasible option to mitigate or prevent age-related diseases in menopausal stages (peri and post)[68,69,70]. This led to large RCTs to study the effects of postmenopausal HT in the 1990s[4]. **Table 1** summarizes the timeline of postmenopausal HT and important studies on HT effect.

The Heart and Estrogen/Progestin Replacement Study I and II (HERS I and II) was a randomized, double-blinded, placebo-controlled clinical trial designed in 1993 to test the efficacy and safety of oral estrogen plus progestin (CEE and MPA) therapy for prevention

of recurrent coronary heart disease (CHD) in postmenopausal women[71]. Follow-up results from this trial suggest that postmenopausal HT does not protect older women with heart disease against further progression of CHD[72].

The Women's Health Initiative Hormone Therapy (WHI-HT) trials, also initiated in 1993, were designed to investigate strategies for the prevention and control of the most common causes of morbidity and mortality in postmenopausal women[73]. One of its primary objectives was to examine the role of HT in CHD in women without CHD. The WHI-HT trials stopped earlier than expected (planned date of completion in 2007) due to the greater incidence of CHD, breast cancer, stroke and blood clots in HT groups versus placebo[74]. Results from WHI-HT trials on increased risks for these incidents have led to lower use of HT among postmenopausal women[75]. Implications for the epidemiology of dementia, and for the occurrence of cardiovascular diseases and stroke in women led to investigations on the aspects of cognitive function[76].

Table I. Timeline of important studies on hormone therapy effect

Hormone Therapy Timeline	
1941	**Premarin**, conjugated equine estrogen (CEE) tablets, for treatment of menopausal symptoms becomes available for clinical use and was widely exported from Canada.
1942	FDA approves **Premarin**, made from pregnant horse urine.
1992	**Premarin** marks number 1 prescribed drug in the U.S.
1993	**HERS** and **WHI-HT** trials launch to test potential benefits of postmenopausal hormone therapy (HT) for prevention of coronary heart disease (CHD) in women.
1995	**WHIMS** starts as an ancillary study to the WHI-HT trials to test the effects of postmenopausal HT on brain cognition.
1996	**SWAN** observes endogenous E2 levels and menopausal transition in middle-aged women with no link to cognitive deficits.
1997	**Premarin** sales reach $1 billion.
1998	Both **HERS** and **WHI-HT** studies find no benefits of postmenopausal HT on heart disease prevention.
2000	**EMS** finds positive effects of E2 on short-delay verbal recall in postmenopausal women with intact and better cognitive performance.
2002	**WHI-HT** stops its estrogen+progestin trial due to adverse effects of heart disease, breast cancer, stroke and blood clots in treatment group vs. placebo.
2003	FDA recommends short-term postmenopausal HT with low doses for needed treatment goals in menopausal women.
2004	**WHI-HT** estrogen-alone trial terminates earlier than expected due to no reduction of the risk of heart disease.
2004	**WHIMS** finds a harmful effect for HT regimens on the brain.
2004	**ELITE** finds neutral effects of the initiation of HT in early versus late menopause; critical window (timing) hypothesis did not inform about AD risk in women.
2005	**KEEPS-COG** finds no cognitive effects of HT routes (oral CEE vs. transdermal E2) in recently postmenopausal women.

The WHIMS launched in 1995 was an ancillary study to the WHI-HT trials[7]. The goal of the WHIMS was to investigate the effects of HT on the risk of probable dementia and cognitive impairment and on changes in global cognition over time in older

postmenopausal women. Primary analysis of the WHIMS included a total of 7,479 women, aged 65-80 years, who were randomly assigned to hormone regimens of oral CEE-alone and CEE+MPA (for women with uterus) or matching placebo followed by annual assessments of global cognitive function and adjudicated cognitive status (no cognitive impairment, mild cognitive impairment, or probable dementia)[6,8]. If a woman was currently on HT during recruitment, she was required to cease use for at least 3 months prior to enrollment. Thus, the WHIMS tested the effect of either first-time initiation or re-initiation of HT on cognitive outcomes. The primary aim of the WHIMS was to test whether the incidence of dementia would differ between women randomly assigned to HT regimens or matching placebo. The initial results from the WHIMS showed that the incidence of dementia was markedly higher among women who had been assigned to active HT (hazard ratio=1.76; p=0.005), with no significant differences between CEE-alone and CEE+MPA[6,8]. Mean global cognitive function scores were found to be significantly lower among women assigned to active HT, with no significant differences between hormone regimens. Although WHI-HT trials were stopped earlier (CEE+MPA in 2002; CEE-Alone in 2004) than planned, follow-up studies continued.

Subsequent follow-up of the cohort demonstrated that these differences on cognitive function between intervention groups were sustained for years after study medications were terminated[10]. An open question remained on whether the administration of HT at the time of menopause, as for the treatment of menopausal symptoms, would have adversely affected women's brains.

The WHIMS in younger women (WHIMSY) studied the effect of HT on cognitive functioning when women were aged 50-55. Results subsequently showed that random

assignments to HT, compared with matching placebo, in recently menopausal women did not produce long-term alterations in cognitive functioning, as assessed an average of 7 years after cessation of treatments[10].

The Study of Women's Health Across the Nation (SWAN) initiated in 1996 was an observational study investigating the menopausal transition and its effects on vasomotor symptoms, sleep, psychological symptoms, cognitive performance, urogenital and sexual health, cardiovascular and cardiometabolic health, bone health, and physical function in middle-aged women[77]. The SWAN study found that changes in cognition were related to normal aging rather than differing levels of E2. This suggests that loss of E2 in the early stages of menopause may not be linked to cognitive decline.

Previous studies support estrogen effects on cognitive performance including verbal learning and memory, working memory/attention, spatial abilities, and visual memory[78,56]. The Estrogen Memory Study (EMS), a randomized double-blind trial, primarily focused on the effects of HT on verbal memory in older women (age >60)[79]. The EMS data suggested that older women with intact or better cognitive performance on the California Verbal Learning Test (CVLT) benefited more from E2 over 2 years than women with poor cognitive performance. This indicates that E2 may exert its neuroprotective effects on women without cognitive decline, but not in women with existing reduced cognitive performance. These findings suggest that the positive effects of E2 are seen in healthy conditions. In this trial, the HT regimen was cyclical E2+Norethindrone (a form of progesterone) and E2-alone. The EMS supported evidence on the beneficial type (E2 over CEE) and the timing of HT initiation. Together with the EMS and earlier findings suggested a “window of opportunity” for HT[80].

The Early versus Late Intervention Trial of Estradiol (ELITE) study was designed to test the critical timing hypothesis for cognitive aging in early (within 6 years) and late (≥10 years) groups of menopausal women[81,82]. The main cognitive outcomes included verbal memory, global cognition, executive functioning and visual memory. The results from ELITE showed neutral (neither improvement nor harms) effects for E2 on cognitive performance in both early and late menopausal groups, regardless of time since menopause, with no significant change.

The Kronos Early Estrogen Prevention Study (KEEPS) was a 4-year randomized, placebo-controlled to investigate the initiation of HT on cardiovascular disease in early postmenopausal women aged 42-58 years old[83]. The KEEPS-Cognitive and Affective Study (KEEPS-COG) was an ancillary study to KEEPS to test the initiation routes of HT (oral CEE or transdermal E2) in recently postmenopausal women (age <60) on global cognition[84]. Findings from this study suggested that HT was neither beneficial nor harmful on global cognition, but raised gaps in understanding the effects of women's metabolic health and HT[83].

The Effects of Postmenopausal Hormone Therapy on Brain Structure and Metabolism

In addition to HT effects on cognition, multiple studies, including some of the large trials already described, investigated changes in brain structure and function in response to HT. Brain magnetic resonance imaging (MRI) was conducted in over 1,400 women from the WHIMS several years following termination of WHI-HT medication to explore potential mechanisms underlying the adverse effects of therapy[9]. This research found that women assigned to HT compared with placebo had significantly smaller brain volumes even after the termination of HT regimens[9]. The associations between decreased brain

volumes and the earlier results from WHI-HT suggested potential detrimental effects of HT on brain structure among these women. Important clues to why HT may be associated with smaller brain volumes have emerged with the discovery that its adverse effects were greatest among women who had T2D at baseline or who developed the disease during follow-up[1,2].

Moreover, recently reported results from the KEEPS study highlight the potential importance of timing, duration, and route of delivery for HT, and also show that the effects of HT on different brain volumes[83,85]. This study suggests that the methods of estrogen delivery (oral CEE versus transdermal E2) may alter the effects of HT on changes in brain structure. The short-term effects of HT may differ from long-term effects and influence on different brain structures. Kantarci et al. (2018) observed greater changes in ventricular volumes in menopausal women who received oral CEE compared to placebo after 4 years[85]. However, HT with transdermal E2 showed preserved prefrontal cortex volumes more than placebo over 7 years. Additionally, women who received transdermal E2 had lower levels of Aβ on PET imaging after 7 years, particularly in APOE ε4 carriers compared to placebo.

Further evidence on functional neuroimaging and metabolic health adds more information about the effects of HT on the brain. Recent studies showed that peri- and postmenopausal women (aged 40-60 years old) exhibit reduced brain glucose metabolism on 18F-fluoro-deoxyglucose (FDG)-Positron Emission Tomography (PET) and mitochondrial function measured by cytochrome c oxidase (COX) activity[86,87]. This suggests that shifts in E2 levels and actions through midlife menopausal transition may inform late-life response to E2 on brain metabolism and neurodegenerative processes (e.g.,

oxidative stress). For example, preclinical research showed that age-related decline in ovarian E2 was associated with a shift from an aerobic glycolytic to a ketogenic phenotype in the female mouse brain[88]. Human brain glucose hypometabolism and mitochondrial dysfunction may be due to this energy source shift in the brain. It is crucial to understand age-related effects of E2 via bioenergetics system in humans.

Recent investigations into sex-specific pathophysiological mechanisms behind ADRD risk have implicated reproductive history, metabolic health and HT use in late-life incidence of neurodegenerative diseases such as AD[89,90]. HT used in previous studies had various forms, doses and regimens (with or without progestin), making it difficult to interpret the consequences of estrogen treatments. However, further evidence on HT suggests that T2D, a metabolic disorder, may play a key role on detrimental effects of postmenopausal HT in older women[1]. It is possible that T2D and E2 may interact through the bioenergetics system to lead to a harmful effect on the brain. In addition to these studies, healthy cell bias (HCB) theory of estrogen actions supports the beneficial effects of HT on glucose metabolism and mitochondrial function in the brain when neurons are disease-free[91]. The effects of T2D on brain metabolism may explain the harmful relationship between T2D and HT in older women.

The adverse effects of HT on neurodegenerative and cognitive processes in older women may be related to alterations of energy metabolism in the brain. The HCB theory hypothesizes that estrogen effects depend on the metabolic health of the E2-targeted cells[91,92]. In healthy hippocampal and cortical neurons, glucose is the main substrate for energy and estrogen actions on glucose-enhancing properties in these neurons are regulatory and protective[93]. E2 activates cell signaling via ERs on the neurons and

enhances glucose uptake, aerobic glycolysis, tricarboxylic acid cycle (TCA)-coupled oxidative phosphorylation and ATP generation in mitochondria. In parallel, E2 increases antioxidant defense and antiapoptotic mechanisms. However, when cells are challenged by free radicals, stress, aging or disease, the brain may shift to an alternative energy use such as ketone bodies to meet its heavy metabolic demands[94]. This proposed model of the bioenergetics alterations in energy metabolism over time (age) and disease progression (ADRD) suggest that relative contribution of glucose declines, while ketone bodies and other fuels increase. Ketone bodies are produced from fatty acids in the liver and can be used as a secondary energy substrate by cells under stressed conditions such as ADRD burden[94,95]. Insulin resistance in T2D may promote the development of ADRD through a variety of mechanisms, including a shift in energy metabolism. HT may negatively affect this bioenergetic shift in postmenopausal women. In this energy source switch, estrogen's glucose-enhancing effect may lose its activity. Estrogens are known to have suppression effects on ketone bodies. The suppressive role of estrogens on ketone body metabolism may deprive cells from this alternate energy substrate on the brain. This scenario poses a risk to brain health and can help answer questions toward the interaction between E2 and T2D, which may link to ADRD pathologies.

The Interaction between Estradiol and Type 2 Diabetes on ADRD Risk

Recent clinical evidence implicated the interaction of T2D and relatively high endogenous E2 levels in postmenopausal women not taking HT with a 14-fold increased risk for dementia, compared with women with low levels of E2[3]. Additionally, re-evaluated WHIMS data showed that the harmful effects of postmenopausal HT on the brain were

driven primarily by women with T2D[1]. These findings suggest that old age and T2D contribute to loss of E2 benefits.

In addition, a study using diabetic, OVX female rats found that E2 replacement in rats exposed to transient forebrain ischemia was associated with a much more severe ischemic brain damage, widespread cell loss and greater neurologic impairment compared to non-E2 treated OVX and intact female rats[96]. The study suggests that benefits of E2 may be diminished by T2D. However, E2 interacting with diabetes caused more damage on the brain than E2-deficiency in this study. It may be still possible that diabetes prevents protective E2's actions toward neurodegeneration. However, mechanisms of metabolic and neuronal activity in the female rat brain such as glucose metabolism are undetermined in this study.

While accumulating evidence suggests that E2 in the presence of T2D may elevate risk to the brain in the postmenopausal phase, some evidence suggests that HT improves impaired glucose metabolism and poor cognitive performance seen in midlife women with T2D[97]. This evidence provides support for the timing hypothesis of HT which suggests early initiation of HT being beneficial versus late-life initiation of HT being harmful on the brain as seen in most RCTs[98,99]. Therefore, both T2D and E2 may contribute to a harmful metabolic effect only in older women.

The mechanisms underlying beneficial versus harmful effects of E2 in T2D remain controversial. More evidence is needed to identify metabolic and functional pathways of E2 actions in regards to T2D in women with old age.

Chapter 2

FEMME STUDY

Results from the earlier RCTs and observational studies have been both influential and controversial on HT and the benefits of E2. However, the controversy for E2's effects on the brain may be resolved by an understanding of the HCB of E2 actions supported in animal models[91], and by the current Female Estrogen Menopause Mind and Energy (FEMME) study testing this theory in humans.

The primary aim of the FEMME study is to test the premise of the HCB theory of estrogen actions *in vivo* in humans. The FEMME study proposes to test the role of estrogenic exposure in postmenopausal women with T2D by using 11C-Acetoacetate/18F-Fluorodeoxyglucose (AcAc/FDG) dual-tracer positron emission tomography (PET) to assess whether short-term (8 weeks) transdermal E2 therapy affects the brain uptake of glucose (on FGD) and ketone bodies (on AcAc) differently in women with and without T2D. The dual-tracer PET imaging technique has been successfully used to assess difference in ketone body and glucose uptake in healthy adults and older adults with cognitive impairment[100,101]. The primary hypothesis of the FEMME study is that estrogens (mainly, E2) affect brain metabolism of glucose and ketone bodies differently in women with and without T2D, in accordance with the HCB theory of estrogen action.

The secondary aim of this study is to assess cognitive effects of short-term transdermal E2 treatment between the comparison groups. The secondary hypothesis is that increased levels of E2 after study treatment alter cognitive performance differently in women with T2D than those without T2D.

This thesis focuses on the addition of a Face-Name Associative Memory Exam (FNAME) fMRI paradigm to investigate associative memory-related brain connectivity patterns in this cohort. The hypothesis is that increased levels of E2 (by the 8-week HT) may alter connectivity in associative memory-related brain networks differently in women with and without T2D, and may improve FNAME performance in women without T2D.

FEMME Study Design

This is a single-site study of Wake Forest University School of Medicine, with two comparison groups: women with prediabetes/T2D (n=10) and women without T2D (n=10) between the ages of 60 and 80.

The criteria for eligibility to the FEMME study for all women are: 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) normal results on recommended healthcare screenings (e.g., mammogram, pap smear, colonoscopy); 4) BMI 20-35 kg/m^2; and 5) no evidence of dementia or mild cognitive impairment (MoCA: Montreal Cognitive Assessment score >25). Women with diabetes must have either physician diagnosis of T2D, or, per American Diabetes Association criteria that show 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 show elevated blood glucose using any of these measures at more than one time. Eligible women with prediabetes must have a current HbA1c value in the prediabetic range of 5.7-6.4 or higher.

Women are excluded from participation in this study if they have: 1) use of hormone replacement therapy within the past 3 months; 2) history of renal, heart, liver, or neurological disease; 3) head injury with loss of consciousness in the past 5 years; 4)

chronic pain, anxiety or depression; 5) presence of medical conditions that might contraindicate estrogen use (e.g., unexplained vaginal bleeding, history of reproductive tissue cancer, thrombosis); 6) current use of insulin, metformin, 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) or any other drug or medication judged by the study physician to affect safety or research outcomes of interest; 7) involved in another research study; 8) contraindications for MRI or PET scanning; 9) current smoker; or 10) reporting a level of radiation exposure in the past year that would cause radiation levels to exceed recommended limits if the person participated in this study. As the FEMME study targets the effects of estrogen on the brain in postmenopausal stage, men are excluded.

Participants

The FEMME study was temporarily suspended on March 18, 2020 due to Coronavirus Disease 2019 (COVID-19) pandemic and restrictions on human research. The target sample size of 20 postmenopausal women (n=10 with T2D/prediabetes, n=10 without T2D/prediabetes) aged 60-80 years old will be recruited from the region of Forsyth County, North Carolina. Six participants have been enrolled and completed baseline visits (n=2 with prediabetes, n=4 non-T2D) before study suspension date. One participant was withdrawn after the baseline AcAc/FDG PET visit due to blood clotting issues. The remaining five women in the study completed neuroimaging, cognitive and metabolic testing at baseline (before E2 treatment). One non-T2D completed baseline and post-E2 treatment visits. Follow-up data for four participants were lost due to temporary study

suspension. Re-enrollment of the remaining participants is anticipated when the study resumes.

Study Intervention

Participants received 0.075 mg/day of transdermal E2 delivered via a Climara® patch for 8 weeks. Participants were instructed to change their E2 patch every week and received weekly phone calls from study staff to confirm the patch was changed and to monitor for side effects. Women with an intact uterus received an additional 2 weeks of combined estrogen and progestin administration after completing all study visits to reverse the effects of E2 in the uterus. Serum E2 levels were measured 48-96 hours (2-4 days) after administration of the second patch to titrate levels as needed to attain a circulating level of 50-100 pg/ml for each woman. The intervention was administered as an experimental manipulation, not treatment for menopausal symptoms.

Study Assessments

Brain MRI scans, dual-tracer AcAc/FDG PET imaging, cognitive testing, and blood sampling were conducted before and after E2 administration. This thesis project focuses on MRI scanning including two FNAME fMRI sessions, and cognitive performance before and after E2 treatment.

The FNAME is sensitive to detect behavioral and functional changes related to associative-memory performance in non-demented elderly adults, as described in previous studies[102,103,104]. FNAME fMRI paradigms used in previous studies assessed associative memory encoding only inside the scanner due to the length of the fMRI design (block design or event-related design) with multiple runs[103,105]. Unique to the current study, the FNAME fMRI paradigm was modified to assess E2-related processes including both

encoding (learning) and retrieval (memory) sessions in the scanner and designed as a continuous task with reduced run times (1 run for each task). The FNAME task has been shown to detect early changes in cognitive functioning in healthy older adults[106]. This task was added to the FEMME study due to its sensitivity to hippocampal activation and changes in E2 levels in cognitively normal older adults, and the reliability to potentially test study interventions in "Proof of Concept" clinical trials as suggested by Putcha et al. (2011) [105,106].

The cognitive testing battery was adapted from the WHI's Study of Cognitive Aging (WHISCA) study trial due to its sensitivity to the effects of estrogens/HT and comprehensive structure to detect age-related changes in memory and other cognitive functions demonstrated in non-demented individuals[78]. The cognitive tests include assessment of verbal knowledge, fluency and memory, figural memory, attention and working memory, spatial abilities, and fine motor speed.

MRI Scanning Sessions

The MRI scan visit consists of a 60-min MRI scan, fasting blood draws, healthy snack, and a 90-minute cognitive testing session. The MRI acquisition includes T1-weighted structural, T2-weighted Flair, resting-state fMRI, FNAME task-based fMRI and diffusion-weighted (NODDI-DTI) MRI sequences. Prior to the MRI scan, participants are informed about the FNAME task and practice a short computer version of this task. During the MRI acquisition session, participants perform the FNAME task while in the scanner.

The FNAME fMRI paradigm was created to assess E2-related encoding (learning) and retrieval (memory) sessions in the scanner using the E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). The FNAME stimuli consist of a digital color photograph

of an unfamiliar face presented against a black background with a fictional first name printed in white underneath the face (face-name pairs). A total of 70 face-name pairs are shown. Each face-name pair is presented only once during the experiment for 5 seconds. The decision to select 70 face-name pairs with 5-second duration for each was based on approximation of previous studies and the behavioral test runs of these stimuli with volunteers. The fixation stimulus is a white fixation cross (+) centered on a black background presented in the beginning of the experiments. The instructions for the experiments are given verbally outside the scanner prior to beginning. Stimuli and written instructions are electronically presented using E-Prime onto a rear projection screen where participants can view the screen through a mirror attached to the head coil. Each session takes about six minutes to complete. The modified version of the FNAME task has two parts to be performed while in the scanner: the encoding and the retrieval sessions.

The encoding session is the learning phase of the task in which participants are presented face-name pairs and instructed to remember them later on (**Figure 1**). While participants study the face-name pairs, they are also asked to decide whether the assigned name fits the face or not, within 5 seconds for each face-name pair. They are instructed to respond with a button press using an MRI-compatible button box whether the name "fit" (button press with right pointer finger) or "did not fit" (button press with right middle finger). After the encoding session, two, study-related MRI modalities are conducted: resting-state fMRI and NODDI-DTI. This creates a 15-minute delay between the encoding and retrieval sessions. Although the retention interval of delayed recall remains controversial, most memory-related exams require a range from 15-30 minutes in delayed recall.

The retrieval session is the recall phase in which participants are asked to recall the name that goes with the face shown in the encoding session (**Figure 1**). Study participants indicate their correct answers with the same MRI-compatible response device. They are instructed to indicate their response by right pointer-finger button press, if the correct name is on the right. They respond with a right middle-finger button press, if the correct name is on the left.

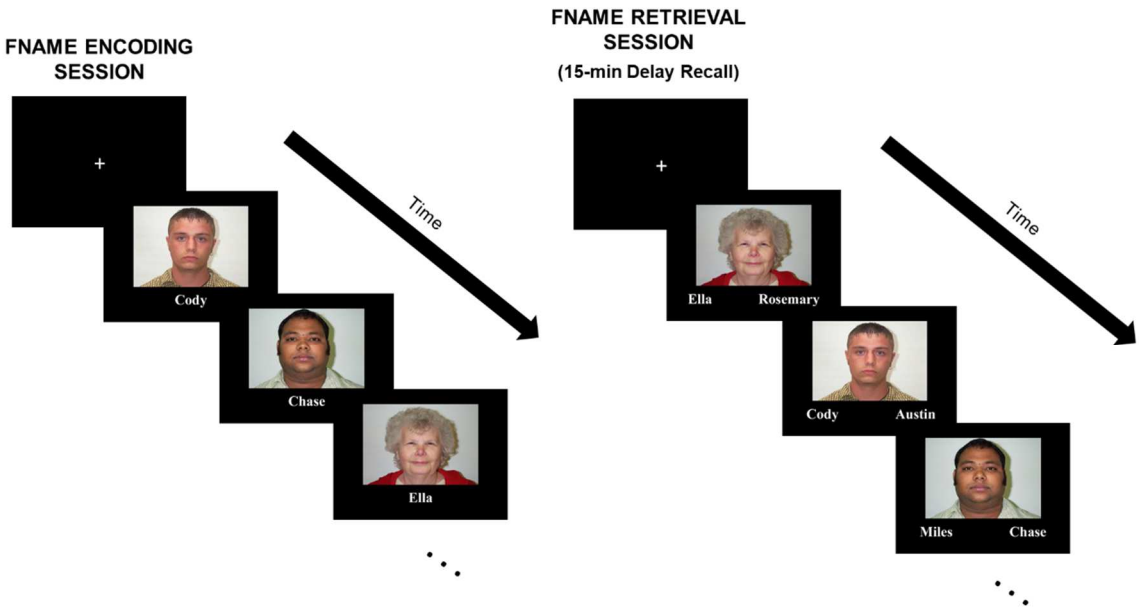

Figure 1. The stimuli of encoding and retrieval sessions of the FNAME. Face-name pairs are presented sequentially for 5 seconds each. A total number of 70 face-name pairs are shown in each session. The order of the stimuli in the retrieval session is different than the encoding session.

Post-Scan Testing

The post-scan stimuli consist of the same face images on a black background now shown with two words printed in white underneath the face: High and Low (**Figure 2**). Participants are asked to indicate whether they have high or low confidence on the correct choice they make in the retrieval session to determine successful encoding. A correct answer at retrieval session with high confidence at post-scan indicates successful encoding of the task.

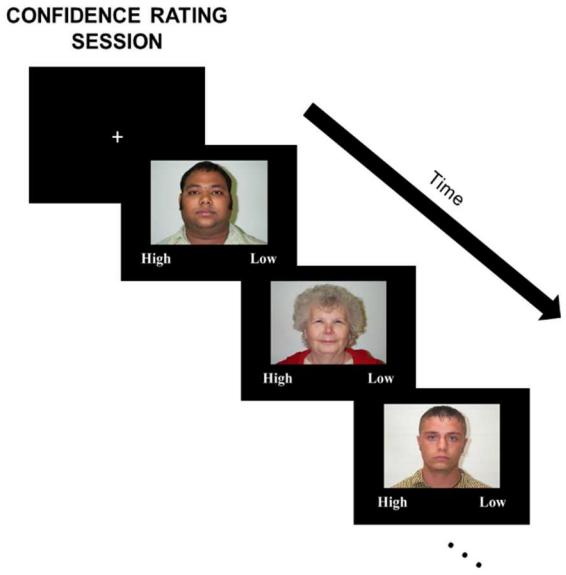

Figure 2. The stimuli of confidence rating session of the FNAME. Duration and the number of face-name pairs presented are the same as encoding and retrieval sessions, except the order of the stimuli (randomized).

Cognitive Testing

After the imaging session, fasting blood draws are collected for serum estrogen, cortisol, glucose, and insulin levels, and participants are given a healthy snack prior to the cognitive testing. After the blood sample collection and the snack, participants are asked to complete a 90-minute battery of cognitive tests. The tests are given orally on paper. The cognitive testing battery include: Vocabulary test for verbal knowledge, California Verbal Learning Test 3rd edition (CVLT-3) for verbal memory, Benton Visual Retention Test (BVRT) for figural memory, Card Rotations for spatial ability, Finger Tapping for fine motor speed, Letters F-A-S for verbal fluency, Fruits and Vegetables for semantic fluency, Prospective Memory Tests for everyday memory, and Number Span: Forward and Backward for attention/working memory.

Proposed Analyses of Brain Networks and Cognitive Performance

A growing body of literature in network science suggests that graph theoretical analysis of brain networks is an invaluable tool for neuroimaging analysis and advances our understanding of the brain's complex structural and functional topology[107]. Network science is an interdisciplinary field, which treats the brain as a system of nodes representing brain regions or voxels, and edges representing the structural or functional connection between these regions or voxels. Importantly, brain networks can provide a fundamental understanding of aging, ADRD, T2D and HT that affect brain's functional and cognitive outcomes. **Figure 3** represents a proposed conceptual model regarding these factors and functional brain connectivity/networks.

The modified FNAME task in this study was designed to assess brain networks extracted from fMRI data. Previous studies using the FNAME paradigm focused on whole-

brain and hippocampal activation of this task in event-related or block designs with multiples runs[102,105]. The proposed experiment of the FNAME paradigm in this study is a continuous task (continuous activation), suggested in brain networks studies[108]. This thesis work proposes to determine whether the short-term administration of transdermal E2 affects community networks differently during encoding and retrieval processes in postmenopausal women with and without prediabetes/T2D. The vertices in networks are often found to cluster into tightly knit groups with a high density of within-group edges and a lower density of between-group edges. This so-called community structure is essential for understanding network organization and topology. HT/E2 may affect community networks related to memory performance. Therefore, the goal of this FNAME fMRI experiment is to identify functional connectivity patterns in associative-memory performance on fMRI before and after transdermal E2 treatment, and conduct a comparison analysis of the groups (non-diabetic vs. prediabetic/T2D). To achieve this objective, a Matlab (R2016b, The Mathworks, Natick, MA) toolbox can be used to generate brain networks and to conduct multivariate analysis on community networks, as previously described[109].

Additionally, this work focuses on cognitive effects of short-term E2 treatment in FEMME study groups. The proposed statistical analyses are Wilcoxon signed rank tests and correlation tests in RStudio (RStudio Team 2015, Boston, MA).

The 2-sample matched pairs Wilcoxon Signed Rank Test (2-s matched pairs WSR) will be used to compare the means of pre- and post-E2 levels of study groups to each other. My effect of interest is the difference variable after 8-week 0.075 mg E2 manipulation. I will use this same approach for pre- and post-cognitive test scores to look at the difference

in verbal memory, figural memory, attention and working memory, spatial abilities, and fine motor speed performance for significance.

Correlation will be used to evaluate the associations between mean differences in estrogen levels (post E2-pre E2) and cognitive scores (post E2 scores-pre E2 scores) for cognitive effects of short-term E2.

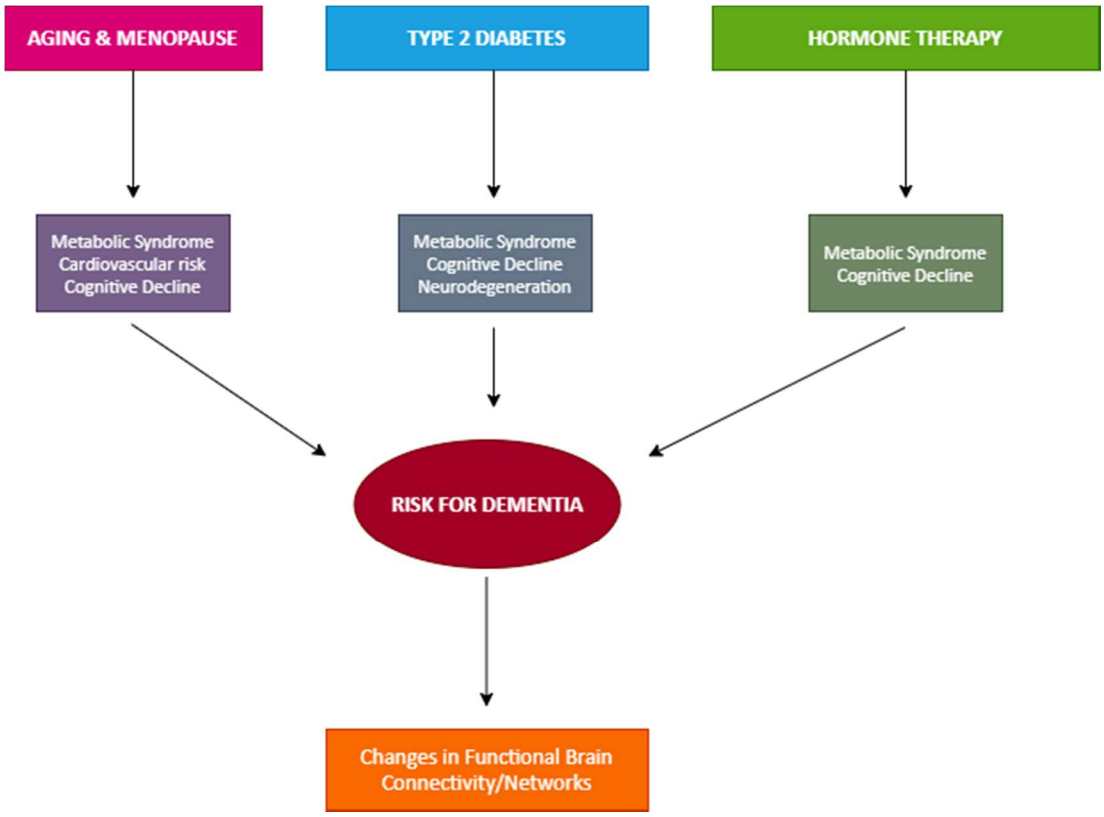

Figure 3. A conceptual model for aging, diabetes, and hormone therapy on dementia risk and changes in functional connectivity. Aging, type 2 diabetes and postmenopausal hormone therapy are independent risk factors for dementia, which may lead to the progression of ADRD. Aging and menopause can affect the risk for metabolic syndrome, cardiovascular disease and cognitive decline. Type 2 diabetes can cause metabolic syndrome, cognitive decline and neurodegeneration. Hormone therapy can impact metabolic syndrome seen in T2D and cognitive decline. These factors may alter functional connectivity in aging and dementia. Changes in functional brain connectivity/networks may indicate an early sign of the development of ADRD.

Chapter 3

SUMMARY AND FUTURE DIRECTIONS

Aging influences changes in metabolic health and brain function and cognition over time. The intersection of age, T2D and HT may pose a unique risk for cognitive impairment and probable ADRD. Results from the earlier preclinical and observational studies support protective effects of mid-life initiation of HT against cognitive decline and metabolic syndrome in women[110,111,112]. However, the large, well-designed clinical trials suggest the opposite, a harmful effect of HT on cognitive outcomes and the risk for dementia in older women[113]. Additionally, this risk seems to be even higher in women with T2D.

Studies on the interaction between HT/E2 and T2D suggest a markedly increased risk for dementia in older women[1,3]. There may be accompanying and/or opposing mechanisms of actions between T2D and E2 on the brain. For example, both E2 and T2D are indicated by the accumulation of AGEs. Chronic hyperglycemia in T2D is accompanied by high levels of AGEs. A study using cellular models suggests that E2 can activate the receptor of AGE (RAGE) gene which would lead to an exacerbation of diabetic pathology[114]. Together, hyperglycemia-induced high AGE and E2-related upregulation of RAGE expression may exacerbate vascular and neuronal tissue damage via oxidative stress and increased inflammation[96,114].

In contrast, recent evidence suggests that E2's metabolic actions may contradict with T2D's metabolic profile on brain glucose metabolism[1,23,115,55,95]. Age-related metabolic disruptions in T2D may cause a switch from glucose metabolism to ketone bodies, an alternative energy metabolism. According to the HCB theory, E2's glucose-enhancing actions may disrupt the ketone body metabolism pathways to deprive cells of

this energy. Consequently, this will lead to neurodegeneration and neuronal dysfunction. The effects of HT in this scenario are harmful on the brain. Support for the HCB theory of E2 actions has been observed in animal studies[91]. Research needs to provide evidence for this theory in women.

The FEMME study proposes to test the HCB theory of E2 in postmenopausal women with and without prediabetes/T2D. To this date, clinical evidence is inconsistent for the benefits of E2 treatment in postmenopausal women. Functional neuroimaging techniques (fMRI and PET imaging) can potentially detect early functional changes in response to estrogenic exposure or HT. Potential neuroimaging outcomes from the FEMME study will inform researchers about the effects of E2 on glucose and ketone body metabolism on AcAc/FDG PET and associative memory-related changes on fMRI in older women with T2D. Most importantly, the FEMME study will demonstrate how these changes differ between women with and without T2D who receive short-term transdermal E2 treatment.

In addition to the primary findings on brain metabolism, the FNAME fMRI paradigm used in this study may prove useful in evaluating the effects of E2 interventions on cognitive performance in aging and early cognitive impairment over short-term periods. Altogether, the future findings from the FEMME study may provide evidence in support of the HCB theory in humans and crucial insights on the benefits/risks of HT use for clinical guidance, especially for older women with T2D.

The interaction of T2D, age and HT may help resolve seemingly contradictory findings in the literature. To our knowledge, the FEMME study is the first comprehensive, proof of concept study designed to identify the effects of E2 on neuroimaging biomarkers

and to test the HCB theory of E2 actions in aged women with and without prediabetes/T2D. The more we know about estrogens action in the brain, the more likely it is to develop designer HTs targeting brain regions vulnerable or susceptible to age-related diseases such as AD dementia.

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57. Berent-Spillson, A. *et al.* Early menopausal hormone use influences brain regions used for visual working memory. *Menopause* **17**, 692–699 (2010).

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CURRICULUM VITAE

EDUCATION

2018-2020 **M.S. Neuroscience**
Wake Forest University; Winston-Salem, NC
Advisor: Christina Hugenschmidt, PhD
Thesis: "The Interaction Between Diabetes and Estradiol in Menopausal Women: Current Reports from the Female Estrogen Menopause Mind and Energy (FEMME) Study"

2017 **Non-Degree Graduate Student**
Indiana University (IUPUI)**;** Indianapolis, IN

2010-2014 **B.S. Biology**
Mugla Sitki Kocman University; Mugla, Turkey

Microbiology and Molecular Genetics Track

2014 **Erasmus$^+$ Student Mobility Programme** (study abroad)
University of Technology and Life Sciences; Bydgoszcz, Poland

RESEARCH EXPERIENCE

2018-2020 **Graduate Research Associate,** The Hugenschmidt Lab
Wake Forest University; Winston-Salem, NC
PI: Christina Hugenschmidt, PhD

- Work on a proof-of-concept study (an R21 funded by NIA) looking at the interaction between diabetes and estradiol on functional MRI (fMRI) with 8-week administration of estrogen therapy in comparison of postmenopausal prediabetic/diabetic and nondiabetic women for Master's thesis
- Create the R21 study brand FEMME (Female Estrogen Menopause Mind and Energy), including title, logo and facilitating flyer construction

- Conduct in-depth literature review for FEMME that modified study assessments to include fMRI task paradigms and women's health questionnaires based upon findings
- Author abstracts and review papers documenting FEMME study design, importance and gaps in menopause and aging field
- Build study forms/questionnaires and manage data on REDCap
- Conduct study visits involving MRI and PET scan acquiring
- Modify and write scripts for neuroimage processing and analysis

2015-2018 **Research Associate,** The Apostolova Lab
Indiana University; Indianapolis, IN
PI: Liana Apostolova, MD MSc

- Conducted research studies on Alzheimer's disease (AD) and other dementias using neuroimaging (MR and PET images), genetic and clinical data methodologies to analyze and identify stage-specific brain imaging and genetic biomarkers for early detection of AD
- Investigated various neuroimaging and statistical techniques to advance current studies such as statistical brain mapping in early-onset and late-onset of AD to monitor disease progress
- Assisted in grant applications (NIA R56) providing results of analyses
- Authored abstracts and research papers documenting the purpose, methods and results of analyses
- Trained incoming laboratory members on research methods used such as data management and neuroimaging and statistical analyses
- Managed extensive databases of interdisciplinary studies and cross-country transfer of thousands of study specimens (blood samples) while maintaining chain of custody and inventorying in specified parameters (temperature, organization)

2015-2018 **Research Associate,** The Perry Lab
Indiana University; Bloomington, IN
PI: Brea Perry, PhD

- Conducted face-to-face survey interviews with participants with cognitive impairment to determine the role of social networks in cognitive status and the trajectory of cognitive decline among older adults
- Authored relating abstracts and papers

2015 **Research Volunteer,** The Tanaka Lab
Indiana University; Indianapolis, IN
PI: Hiromi Tanaka, PhD

- Participated in a molecular genetics research attempting to develop a blood-based assay for early detection of breast cancer based upon specified cell lines (T47D) and telomeric cell-free DNA in plasma
- Performed assays and analysis relative to telomere length, telomere fusion and telomerase activity

2014 **Erasmus$^+$ Student Intern,** The Łukanowski Lab
University of Technology and Life Sciences; Bydgoszcz, Poland
PI: Aleksander Łukanowski, PhD

- Shadowed a research study of fungi (Fusarium sambucinum) growth on potatoes and performed DNA isolation and PCR

2014 **Erasmus$^+$ Student Intern,** The Slawinska Lab
University of Technology and Life Sciences; Bydgoszcz, Poland
PI: Anna Slawinska, PhD

- Shadowed a research study of immune-related gene expression in chickens and performed DNA isolation and PCR

2013 **Summer Research Intern,** The Azhar Lab
Indiana University; Indianapolis, IN
PI: Mohamad Azhar, PhD

- Participated in a research study of mice carrying a knockout allele of Transforming Growth Factor Beta2 (TGFβ2) gene required for valve remodeling during heart development

SCHOLARLY WORKS

Publications

Stage E, Svaldi D, Phillips M, Canela VH, **Duran T**, Goukasian N, Risacher SL, Saykin A, Apostolova LG. Neurodegenerative Changes in Early & Late-Onset Cognitive Impairment with and without Brain Amyloidosis. Submitted on 10/24/2019. Publication for *Alzheimer's Research & Therapy*.

Duran T, Svaldi D, Risacher SL, Stage E, Phillips M, Goukasian N, Hwang K, Woo E, Nho K, West JD, Apostolova LG. Associations between Cortical Thickness and Metamemory in Alzheimer's Disease. Under Revision. Publication for *Alzheimer's & Dementia.*

Stage E, Phillips M, Canela VH, **Duran T**, Goukasian N, Risacher SL, Saykin A, Apostolova LG, for the Alzheimer's Disease Neuroimaging Initiative (ADNI). Early- and Late-Onset Alzheimer's and Suspected Non-Alzheimer Pathophysiology in the A/T/N Framework. Submitted on 06/27/2019. Under Revision and Resubmission for publication in *Neurology*.

Apostolova LG, Risacher SL, **Duran T**, et al. Associations of the Top 20 Alzheimer Disease Risk Variants with Brain Amyloidosis. JAMA Neurol. 2018 Jan 16. doi:10.1001/jamaneurol.2017.4198. *Nature Reviews*.

Stage E*, **Duran T***, Risacher SL, et al. The Effect of the Top 20 Alzheimer Disease Risk Genes on Gray-Matter Density and FDG PET Brain Metabolism. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2016;5:53-66. doi:10.1016/j.dadm.2016.12.003. *Indicates co-first authorship. *ALZFORUM Reviews.*

Presentations

Duran T. The Interaction between Diabetes and Estradiol in Postmenopausal Women: Current Reports from The Female Estrogen Menopause Mind and Energy Study. Oral presentation for the Neuroscience Tutorial Course at Wake Forest School of Medicine. April 10, 2020.

Duran T, Baker LD, Espeland MA, et al. FEMME (Female Estrogen Menopause Mind and Energy) study: The Interaction between Diabetes and Estradiol on Human Brain Metabolism in Postmenopausal Women. 1-minute Oral and Poster Presentations (#3 and #4, respectively among the top 10 scoring abstracts) at Women's Health Research Day. April 17, 2019, Wake Forest School of Medicine. (school.wakehealth.edu/Women-in-Medicine-and-Science/)

Duran T, Woo E, Svaldi DO, et al. Associations between Cortical Thickness and Metamemory In Alzheimer's Disease. Poster Presentation at Alzheimer's Association International Conference (AAIC) July 21-22, 2018, Chicago, IL, USA.

Duran T, Risacher SL, Goukasian N, et al. The Effects of the Top 20 Alzheimer's Disease Risk Genes on Brain Atrophy. Oral Presentation at Alzheimer's Association Imaging Consortium (AIC) July 23, 2016, Toronto, ON, CA.

Apostolova LG, Goukasian N, Risacher SL, **Duran T**, et al. Alzheimer's Disease Risk Genes Can Predict Brain Amyloidosis. Poster Presentation at Alzheimer's Association International Conference (AAIC) 2016 (July 24) in Toronto, ON, CA.

Apostolova LG, Risacher SL, **Duran T**, et al. The Effects of the Top 20 AD Risk Variants on Brain Amyloidosis and Neurodegeneration. Poster Presentation at Alzheimer's Association International Conference (AAIC) 2016, July 24-25 in Toronto, ON, CA and at Big Data Neuroscience Workshop: Organized by the Advanced Computational Neuroscience Network (ACNN) September 8-9, 2017, Bloomington, IN, USA.

Abstracts

Sanjay AB, Hwang K, Svaldi DO, **Duran T**, et al. Predicting Brain Amyloidosis Using Peripheral Blood-Based Gene Expression and Early Stage Neurodegeneration Biomarkers. Alzheimer's Association Imaging Consortium (AIC) July 13, 2019, Los Angeles, CA, USA. IC-P-051 DOI: https://doi.org/10.1016/j.jalz.2019.06.4894

Stage E, Svaldi DO, Phillips M, **Duran T**, et al. Early and Late-Onset Alzheimer's Disease and Suspected Non-Alzheimer Pathophysiology within the A/T/N Framework. Alzheimer's Association Imaging Consortium (AIC) July 13, 2019, Los Angeles, CA, USA. IC-P-181 DOI: https://doi.org/10.1016/j.jalz.2019.06.4296

Svaldi DO, Yan X, Patania A, Sanjay AB, **Duran T**, et al. Evaluating the Utility of Homological Structural MRI Features and a Kernel-Based Learning Framework for Prediction of MCI. Alzheimer's Association Imaging Consortium (AIC) July 13, 2019, Los Angeles, CA, USA. IC-P-129 DOI: https://doi.org/10.1016/j.jalz.2019.06.4243

Duran T, Baker LD, Espeland MA, et al. The Interaction between Diabetes and Estradiol on Human Brain Metabolism in Postmenopausal Women: FEMME (Female Estrogen Menopause Mind and Energy) Study. Women's Health Research Day (WHRD) April 17, 2019, Wake Forest School of Medicine, Winston-Salem, NC. school.wakehealth.edu/Women-in-Medicine-and-Science/

Duran T, Woo E, Svaldi DO, et al. Associations between Cortical Thickness and Metamemory in Alzheimer's Disease. Alzheimer's Association International Conference (AAIC) July 21-22, 2018, Chicago, IL, USA. IC-P-047 DOI: https://doi.org/10.1016/j.jalz.2018.06.2111

Perry BL, McConnell W, Finley E, **Duran T**, et al. Social Networks and Cognitive Performance in Older Adults with Normal Cognition, Mild Cognitive Impairment, and Mild Alzheimer's Disease. Alzheimer's Association International Conference (AAIC) July 2017, London, UK. P1-552 DOI: https://doi.org/10.1016/j.jalz.2017.06.568

Stage E, Phillips M, Canela VH, **Duran T**, et al. Comparing Imaging Phenotypes of Amnestic Early versus Late-Onset Amyloid-Negative Mild Cognitive Impairment and Dementia ADNI Subjects. Alzheimer's Association International Conference (AAIC) July 2017, London, UK. IC-P-216 DOI: https://doi.org/10.1016/j.jalz.2017.06.2592

Canela VH, Stage E, Phillips M, **Duran T**, et al. Differences in Brain Metabolism in Early and Late Onset Alzheimer's Disease and Suspected Non-Alzheimer's

Pathophysiology. The 69th American Academy of Neurology (ANN) Annual Meeting, April 2017, Boston, MA, USA. P4.099.

Stage E, Phillips M, **Duran T**, et al. Brain Atrophy in Early and Late Onset Alzheimer's Disease and Suspected Non-Alzheimer's Pathophysiology. The 69th American Academy of Neurology (ANN) Annual Meeting, April 2017, Boston, MA, USA. P4.098.

Apostolova LG, Risacher SL, **Duran T**, et al. Examining the Effect of the Top 20 AD Risk Variants on Brain Amyloidosis, Structural Atrophy and Metabolism. Alzheimer's Association International Conference (AAIC) July 2016 in Toronto, ON, CA. IC-P-059
DOI: https://doi.org/10.1016/j.jalz.2016.06.089

Stage E, **Duran T**, Risacher SL, et al. Association of FDG-PET Brain Metabolism with Alzheimer's Disease Risk Genes. Alzheimer's Association International Conference (AAIC) July 2016 in Toronto, ON, CA. IC-P-066.
DOI: https://doi.org/10.1016/j.jalz.2016.06.096

Duran T, Risacher SL, Goukasian N, et al. The Effects of the Top 20 Alzheimer's Disease Risk Genes on Brain Atrophy. Alzheimer's Association International Conference (AAIC) July 25, 2016, Toronto, ON, CA. P2-253
DOI: https://doi.org/10.1016/j.jalz.2016.06.1422

Apostolova LG, Goukasian N, Risacher S, **Duran T**, et al. Regional and Stage-Specific Association of Multiple AD Risk Variants with Brain Amyloidosis. Organization for Human Brain Mapping (OHBM) Annual meeting, June 2016, Geneva, Switzerland.

SOCIETIES & AWARDS

- Travel Fellowship for National Institute on Aging (NIA) Workshop on Cognitive Benefits of Menopausal Hormone Therapy August 15-16, 2019
- The Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART) – Student Member (July 2016 – July 2018)

- Travel Fellowship for the Alzheimer's Association International Conference® (AAIC) 2016
- Registration Fellowship for the Alzheimer's Imaging Consortium (AIC) of AAIC 2016