# National Institute on Aging Workshop on Cognitive Benefits (and Costs) of Hormone Therapy

## Workshop Summary

**Prepared by**

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**Executive Summary**

The National Institute on Aging (NIA) hosted a workshop on August 15-16, 2019, titled "Cognitive Benefits (and Costs) of Hormone Therapy." Investigators from academic and medical research centers and NIH staff reviewed the current state of active research on menopausal hormone therapies and identified gaps in the evidence base.

Clinical trials of hormone therapy have shown relief of menopausal symptoms for perimenopausal women, including vasomotor symptoms, sleep disruptions and mood changes, with no meaningful cognitive benefit or cost when hormone therapy was used for up to five years. However, in post-menopausal women, hormone therapy was associated with detrimental effects on cognition and increased brain atrophy; the adverse effects may be greatest among women who have type 2 diabetes or pre-diabetes at baseline. Also, the effects are modulated by *APOE* ε4 status, which increases the risk for cognitive decline and Alzheimer's disease.

Several recent or ongoing observational studies involving perimenopausal women were reviewed that showed verbal memory worsening during the perimenopause, even after accounting for age at onset. Changes in endogenous estradiol levels during the perimenopause were associated with cognitive changes. In natural menopause, more severe vasomotor symptoms, including objectively measured hot flashes and sleep disturbance, were associated with adverse effects on memory and reduced left hippocampal activity. Women who underwent surgical oophorectomy prior to natural menopause had a steeper slope of cognitive decline if their oophorectomy occurred at age 38-43 compared to women whose oophorectomy occurred at age 44 and older, and hormone therapy mitigated these changes. In both populations, hormone therapy was associated with diminished structural and functional declines associated with menopause. Women with metabolic syndrome or insulin resistance showed steeper trajectories of cognitive decline.

Animal studies indicate that cognitive deficits after surgical menopause can be ameliorated by estradiol administration. Several putative mechanisms of action were suggested. Estradiol treatment increases estrogen receptor alpha in the hippocampus. Direct manipulation of estrogen receptor alpha expression in the hippocampus, via overexpression with viral vectors or receptor blockade via central administration of estrogen receptor antagonist, revealed that estrogen receptor alpha in the hippocampus facilitates better memory in the absence of ovarian or exogenously administered estrogens. Estrogen receptors can also be activated in a ligand independent manner by insulin-like growth factor 1 in the absence of estradiol. Estradiol has been shown to regulate spine density in pyramidal neurons of mice and rhesus macaques. In mice, estradiol's effect on dendritic spine density in the medial prefrontal cortex is reliant upon phosphorylation of the protein kinases ERK and mTOR. In aged monkeys, surgical menopause produces a loss of thin dendritic spines on prefrontal pyramidal neurons, accompanied by deficits in spatiotemporal working memory. Both changes can be remediated by cyclic, but not continuous estradiol treatment. Cyclic administration of estradiol is effective in improving working memory in aged monkeys even if treatment begins more than two years after ovariectomy.

The workshop participants suggested research topics that represent gaps in the knowledge base. Additional basic research would help elucidate molecular and cellular mechanisms and brain circuits by which sex steroid hormones regulate cognitive function throughout the lifespan. Many animal studies suggest cognitive benefit from menopausal hormone therapy, while large, well-designed clinical trials in humans have failed to demonstrate cognitive benefit. Better insight into this apparent paradox could inform the design of future clinical trials. It is important to understand how cognition changes in relation to menopause induced surgically or chemically, in comparison to natural menopause. Fundamental gaps remain about the formulation, dose, and duration of hormone therapy in relation to cognitive and brain aging. It is likely that optimal formulations will differ among women, depending on age, genetic background, reproductive history, and other factors. There was agreement about the need for a life course

perspective to understand how early life exposures, such as oral contraceptive use or hypertensive pregnancy disorder, may modulate the response to menopause and hormone therapy. Larger datasets are needed to study the large number of factors implicated as putative modifiers of treatment effects. Greater harmonization of data is needed across clinical trials and cohort studies of menopausal hormone therapy to support epidemiological studies that use machine learning approaches to investigate genetic and phenotypic factors that interact with hormone therapy.

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The National Institute on Aging (NIA) held a workshop August 15-16, 2019, on Cognitive Benefits (and Costs) of Hormone Therapy. The day and a half of meetings, held in Bethesda, MD., brought together two dozen investigators from academic and medical research centers, staff from NIH, and several observers who were trainees from the research community. The purpose of the workshop was to review the current state of active research on this topic and to identify gaps in the evidence base that limit a clear understanding of the cognitive benefits and costs of menopausal hormone therapy (HT).

## 1. Workshop Welcome, Format, and Goals

The workshop opened with welcoming remarks by Luci Roberts, Ph.D., the workshop moderator and program director in the Behavioral & Systems Neuroscience Branch of NIA's Division of Neuroscience. NIA organized the workshop to survey the state of existing knowledge about HT and cognition in peri- and postmenopausal women, as a basis for identifying research gaps and recommending ways to move the field forward.

A keynote address provided historical perspective on HT use by postmenopausal women. Then, researchers described their work and primary findings in three areas of focus: 1) evidence from studies of menopause and HT on cognition and affect; 2) effects of HT on brain structure and function; and 3) biological risk factors for cognitive aging and dementia. The workshop ended with a roundtable discussion in which the participants discussed research to date and offered recommendations on research directions and priorities.

## 2. SESSION 1: EVIDENCE FROM STUDIES OF MENOPAUSE AND HORMONE THERAPY

### 2.1 Keynote Address: Menopausal Hormone Therapy: A Historical Perspective

Susan Resnick, Ph.D., a senior investigator at NIA's Intramural Research Program, summarized the history of HT for menopausal women. She referred the group to a 2005 paper by Marcia L. Stefanick, Ph.D., in the *American Journal of Medicine*, titled "Estrogens and Progestins: Background and History, Trends in Use, and Guidelines and Regimens Approved by the U.S. Food and Drug Administration." The first widely used drug for treating menopausal symptoms was Premarin, conjugated equine estrogen (CEE) derived from urine of pregnant horses. Manufactured by Wyeth, Premarin was first used in Canada in 1941 for the treatment of hot flashes and other symptoms of menopause, and a drug application was filed with the Food and Drug Administration (FDA) the following year. Use of the drug soared in the U.S., bolstered by endorsements such as a 1966 best-selling book, *Feminine Forever*, by Dr. Robert Wilson, in which he argued that women experiencing menopause should be treated with HT to address "the loss of womanhood and the loss of good health." By 1992, Premarin was the No. 1 prescribed drug in the U.S., and sales approached $1 billion a year in 1997. Menopause, Dr. Resnick said, came to be viewed as "a galloping catastrophe," with estrogens as the cure. The medicalization of menopause created a huge new market, and advertising campaigns featured celebrities such as model Lauren Hutton touting the benefits of estrogens. In the 1990s, medical organizations began recommending estrogens as protective against heart disease. Underscoring the increasing focus on menopause and HTs, in 1995 *Time* magazine featured a cover story titled "The Estrogen Dilemma."

Against this backdrop, in 1993 NIH launched the Women's Health Initiative (WHI), consisting of three clinical trials and an observational study to examine health outcomes in postmenopausal women and including 161,000 women aged 50-79 at baseline. The three overlapping clinical trials tested the effects of HT, dietary modification, and calcium/Vitamin D supplementation. The HT component of the study (27,000 women) entailed two separate clinical trials for women with or without a uterus, each with its own placebo group. Women who still had their uterus also were administered a progestin to oppose the uterotropic effects of estrogens. The HT trials were designed to test the effects of postmenopausal HT on

coronary artery disease (an expected benefit), breast cancer (an anticipated risk), and other conditions such as bone loss, blood clots, and in women with a uterus, endometrial cancer.

Basic science studies using cellular models and studies in animal models have provided evidence of beneficial effects of estrogens on brain structure and function, including neuroprotective benefits, increased cerebral blood flow and glucose metabolism, as well as amyloid processing. Observational studies in humans suggested that estrogen-containing HT decreased the risk for Alzheimer's disease, while small randomized clinical trials found that HT improved memory and attention in women who underwent surgical menopause. WHI ancillary studies—the WHI Memory Study, or WHIMS, and the WHI Study of Cognitive Aging, or WHISCA—set out to investigate whether HT protected against dementia and age-related cognitive decline in women age 65 and older.

Results of the HT trials of the WHI created panic among women and their doctors when it was widely reported that HT increased the risk of harmful effects such as heart attacks, breast cancer, strokes, blood clots, and dementia (the results varied somewhat according to CEE -only or CEE-progestin regimens). Concluding that the "risks outweighed benefits," WHI ended the estrogens-progestin portion of the trials early in 2002 and the CEE-only trials in 2004 due to anticipated lack of benefit and increased risk for stroke. The FDA issued a "black box" warning on estrogen products in 2003, and the use of HT plunged. Once again, the question of HT use dominated the news.

It is important to note, Dr. Resnick said, that the WHI HT trials were not designed to evaluate the effects of estradiol. Rather, the trials were designed to address an important public health question because approximately six million women were using Premarin containing HT prior to the WHI findings, and many physicians continued to recommend initiation of HT for prevention of cardiovascular disease, even in older postmenopausal women. As compared to observational studies showing benefits of HT on cognition, women in the randomized WHIMS and WHISCA studies differed with respect to age and age at initiation of HT. Other variables that may influence effects of HT on cognitive function include specific HT regimens and duration of treatment. Participant follow-up continues in WHIMS, and relevant findings are also available from other studies described at length later in the workshop presentations, including WHIMSY (a component of WHIMS involving younger postmenopausal women), the Kronos Early Estrogen Prevention Study (KEEPS) and the Early Versus Late Intervention Trial with Estradiol (ELITE), which include studies of cognitive endpoints with HT.

In a brief discussion period, Dr. Resnick and other participants offered a number of questions and issues of wide interest in the field: 1) is there a "window of opportunity" during which women are most likely to benefit from HT (which raises the need to study effects during perimenopause as well); 2) how does a "healthy brain" respond to HT differently from one already compromised by conditions such as neurogenerative disease; 3) what role does the apolipoprotein E (APOE) genotype play as a risk factor in cognitive decline; 4) who are the most vulnerable populations that research should focus on;, and 5) how do the effects of HT regimens used in the previous clinical trials compare with bioidentical HT (now increasing, but not regulated).

## 2.2 Evidence on Cognition and Affect

### *2.2.1 Hormone Therapy: Lessons Learned from the Women's Health Initiative Memory Study (WHIMS) and the Action for Health in Diabetes*

Mark Espeland, Ph.D., from the Wake Forest School of Medicine, reported findings from the Women's Health Initiative Memory Study **(**WHIMS), an ancillary study of the WHI HT clinical trials. WHIMS enrolled 7,479 women, aged 65-80, who were randomly assigned to therapy regimens of CEE—with or without the progestin medroxyprogesterone acetate, depending on uterus status—or matching placebo; they were followed annually to assess global cognitive function and level of cognitive status, from normal

to dementia. (If a woman was currently using HT during recruitment, she was required to stop for at least 3 months prior to enrollment; thus, the WHIMS-HT trial tested the effect of either initiation or re-initiation of therapy on study outcomes.) The primary hypothesis of the WHIMS trial was that the incidence of dementia would differ between women assigned to active versus placebo therapy.

As reported in 2004, WHIMS investigators found markedly higher dementia incidence and poorer global cognitive function test scores among the women assigned to HT compared with those assigned to placebo, with no significant differences between hormone regimens. Statistically significant relative decrements in cognitive function were still evident over a decade after use of study HT had been terminated. Brain magnetic resonance imaging conducted on 1,400 WHIMS women to explore potential mechanisms underlying the adverse effect showed the women previously assigned to HT had significantly smaller brain volumes compared with those assigned to placebo. Among women who had been assigned to HT, smaller brain volumes were associated with the development of cognitive impairment, while among women who had been assigned to placebo, development of cognitive impairment was associated with markers of cerebrovascular disease. These findings suggest that the mechanism by which HT increases risks for dementia in older women is linked to an increased rate of brain atrophy.

Important clues as to why HT may increase brain atrophy have emerged with the discovery that its adverse effects were greatest among women who had type 2 diabetes mellitus at baseline or developed the disease during follow-up, i.e. who may have had pre-diabetes at baseline. This finding signaled that HT's adverse effects on cognition in older women may be in part related to alterations of energy metabolism in the brain. Estrogen is known to downregulate metabolic pathways that may be increasingly important when glucose-based energy metabolism is compromised.

The WHIMSY found that random assignment to HT when women were aged 50-55 was not associated with long-term differences in cognitive function after therapy study was terminated.

*2.2.2 Findings from the KEEPS-Cog: Influence of Formulation and Mode of Delivery on Cognitive and Mood Cost/Benefit Considerations*

Carey Gleason, Ph.D., of the Alzheimer's Disease Research Center at the University of Wisconsin–Madison, summarized findings from KEEPS-Cog, an ancillary study of the Kronos Early Estrogen Prevention Study. KEEPS, started in response to evidence from the WHI clinical trials indicating that women receiving HT had an elevated risk for cardiovascular disease, explored the hypothesis that initiation of HT within three years of a woman's last menstrual period would decrease the rate of atherosclerotic plaques. A cognitive and affective study (KEEPS-Cog) was included to investigate cognitive and mood effects, in the face of conflicting reports from WHI and other studies about HT's effects on brain health.

The previous WHI trials showed the detrimental effects in women who were 65 and older—well past menopause. KEEPS set out to determine whether the same effects occurred in younger postmenopausal women. Conducted at nine U.S. academic centers, KEEPS was designed as a 4-year, randomized, double-blinded, placebo-controlled clinical trial to compare HT outcomes from different drug formulations and modes of delivery. Of the 727 women enrolled in KEEPS, 693 participated in the KEEPS-Cog component; they had low cardiovascular risk, received HT within three years of menopause, and were, on average, 52.6 years old. The women were randomized to receive placebos or one of two forms of estrogen—CEE (Premarin; 0.45 mg/d) or a 17β-estradiol patch (Climara; 50 μg/d)—plus 12 days of progestin in the active groups. A battery of assessments was conducted at several intervals over the 4 years to measure cognitive function and mood.

The KEEPS-Cog findings showed that for recently postmenopausal women, HT does not alter cognition, as hypothesized. Of note, the oral CEE formulation improved mood-related symptoms. These findings

have potentially broad implications because 80% of menopausal women report neurological symptoms such as sleep disruption, mood changes, and anxiety or depression. To understand the cognitive effects of HT over the long term, the women will be reevaluated 12 years after randomization in the KEEPS – Continuation study. Dr. Gleason concluded by noting that the findings are not generalizable because the women in the study were a unique group of very healthy women. HT may have mixed effects depending on a woman's individual metabolic health and risk for conditions such as Alzheimer's disease, which raises the importance of more personalized medicine approaches. The KEEPS – Continuation will provide additional findings to further clarify the importance of the hypothesized "critical window" in the menopausal process during which HT can potentially be protective for brain health.

*2.2.3 Menopause and Cognition Using Rat Models: A Trip Down Memory Lane*

Heather Bimonte-Nelson, Ph.D., from Arizona State University–Tempe, explained first how experiments are done to test cognition in rodents (spatial abilities, short-term and long-term memory), then summarized findings of several rodent studies of menopause, HT, and cognition to illustrate developments in the field. Clinical studies such as the WHI and WHIMS sparked many rodent studies to investigate estrogens' and progestogen's roles in brain health and functioning. One early study found that surgically menopausal rats receiving a moderate dose of E2 were better able to handle a high working memory load than rats receiving the vehicle control with no E2 or a low dose of E2. Studies of other tested compounds included one showing that CEE enhanced memory and protected against drug-induced amnesia in ovariectomized rats. Adding progestogens, which is necessary for women with a uterus, complicates the picture because preclinical literature has suggested that E2 treatment enhances cognition while progestins impair it. In fact, rodent work has shown that some chronically administered progestins reverse the beneficial effects of E2 on cognition and related brain markers, such as neurotrophins. These and other results, Dr. Bimonte-Nelson noted, have led to a search for progestins that protect the uterus but do not reverse the memory-enhancing effects of E2, for ways to bypass the need for a progestin by targeting E2 to the brain, and new forms of progestogens localized to the uterus.

Researchers are now modeling various menopausal etiologies and endogenous hormone backgrounds in rats to translationally address questions about HT. The chemical 4-vinylcyclohexene dioxide (VCD) can induce transitional ovarian follicular depletion in rats to achieve a hormonal profile like that of naturally menopausal women. Dr. Bimonte-Nelson and her team have shown that the transition to menopause when in young adulthood is a particularly sensitive time for memory change, as young VCD-treated rats undergoing follicular depletion were shown to perform worse on memory tests than age-matched counterparts treated with a vehicle control that did not induce follicular depletion. Moreover, challenging the widespread dogma that a non-pregnant uterus is "dormant and useless," Dr. Bimonte-Nelson said existing needs in modeling menopause include answering questions about the role of the uterus. It has been shown, for example, that hysterectomy impairs rats' ability to handle an increasing working memory load, and research in women has demonstrated an increased dementia risk after hysterectomy.

Menopause, Dr. Bimonte-Nelson emphasized in conclusion, is an endocrine-brain-aging triad where many paths meet, and that while we have learned much in our field, each discovery leads to more questions. A better understanding of menopause and optimizing HT will be achieved by researchers working together across disciplines and species. Rodents are good models because they allow researchers to study effects across the trajectory of aging over the course of approximately two years, to control pharmaceutical exposures, and to strategically manipulate and induce menopause variants yielding profiles like those in women. The way forward also requires a willingness to challenge dogma, she added, as factors or effects now considered nuances may in fact prove to be critical factors driving efficacy.

*2.2.4 Long-Term Impacts on Cognitive Aging of Short-Term Use of Estrogens: The Importance of Estrogen Receptors*

Jill Daniel, Ph.D., Director of the Tulane Brain Institute, described experiments in a preclinical rodent model of surgical menopause in which rodents are ovariectomized in early middle-age to investigate how short-term use of estrogens may affect cognition over the long term. The work has highlighted the important role of estrogen receptors. Dr. Daniel and her colleagues found that previous E2 treatment for 40 days (roughly comparable to 3-4 years in women) in middle-age rats, started at time of ovariectomy, results in long-term enhancement of memory comparable to that of ongoing treatment. Also, this previous midlife E2 treatment results in lasting effects of increased levels of estrogen receptor alpha (ERα) in the hippocampus. These results are apparent up to 8 months after cessation of E2 treatment, indicating they are likely permanent.

Follow-up studies revealed a causal relationship between the ability of previous midlife E2 treatment to increase levels of hippocampal ERα and enhancement of memory. Results of experiments in which ERα was overexpressed in the hippocampus via viral vectors or blocked via intracerebroventricular delivery of ER antagonist revealed that levels and/or availability of ERα in the hippocampus leads to better memory in the absence of ovarian or exogenously administered estrogens.

Ongoing work is examining mechanisms by which brain ERα can influence memory in the absence of ovarian estrogens. Results to date show that: 1) ER are transcriptionally active in the absence of ovarian estrogens; 2) ligand-independent activation by insulin-like growth factor 1 (IGF-1) provides a mechanism by which ERα is activated and positively impacts the hippocampus and memory in the absence of ovarian estrogens, and 3) IGF-1 and neuroestrogens can synergize to impact ER-dependent transcription in the absence of ovarian estrogens.

In conclusion, Dr. Daniel said that in the preclinical model she and her colleagues are using, more ERα in the hippocampus translates to better cognitive outcomes in aging females. Previous history of HT may be one factor affecting those levels.

## 3. Panel Discussion

The discussion opened on questions about implications of NIH's Sex as a Biological Variable policy on having both male and female animals represented in research. Participants noted that the new policy will affect the budget of research grant applications because larger sample sizes may be needed to achieve sufficient power to detect sex differences. Molly Wagster, Ph.D., a branch chief in NIA's Division of Neuroscience, advised the group that budgets for grant applications should be adjusted accordingly in order to address sex as a biological variable. According to the policy, while both sexes must be included in NIH grant applications, not all studies must be powered to detect sex differences. One participant noted that it is a circular problem, as it is not always clear from the outset whether there may be a sex difference. Another participant pointed out that some studies target basic processes within cells that may not have sex differences, whereas regulation of the expression or response may show sex differences.

Dr. Espeland said that from a study design perspective, looking for a treatment * sex interaction requires a much larger sample size than to detect an overall treatment effect. One way to get around that in looking at variability is to pool data. "We have large sources of data from various large studies, but haven't worked toward pooling it together," he said.

NIA has funded the development of these data resources, providing investigators a huge opportunity for pooling data, Dr. Wagster noted. However, there are many other datasets—such as data from relatively small clinical trials, for example, and studies done many years ago, —that could be highly useful, Dr. Brinton remarked, making a case for an "emancipation of data." Dr. Hohman built on the point made by

Dr. Espeland, noting that for genomics to be a useful tool, much larger sample sizes will be required to consider factors such as lifestyle and environmental exposures that may have a huge influence.

Participants turned to the issue of dosage and duration in HT. It was noted that Studies of Women's Health Across the Nation (SWAN) offers probably the only database in which it is possible to follow cognition from peri- to post-menopause. One interesting finding from SWAN is that the group that consistently shows better cognitive outcomes are women who use HT for a short period. In a reference to Dr. Daniels' work, one participant said a study about 15 years ago suggested that long-term use of estrogen therapy leads to methylation of ERα in the heart. Might the same be true for the brain? Should research consider an ERα mechanism in human studies in terms of duration of use? Another participant observed that nonhuman primate data show that cyclic dosage is the key to preserving estrogen signaling in the brain, and an ERα mechanism could be critical to understanding patterns of whether one might see benefits or not.

Musing about the influence of stress led panel members to discuss the variability in both animal and human studies. Even in rodents subjected to the same conditions every day, with stressors distributed relatively uniformly, there are responders and non-responders. Given the complex problem of variability, how can precision medicine be developed for HT? Further complicating the issue in human work is the intersection of biological variability with variability due to diverse life exposure that could lead to susceptibility or reserve and resilience. Thus, it is important to understand both biologically determined variability and variability due to modifiable factors. Dr. Kantarci said work in her lab is showing a lot of variability in biomarkers, and the researchers are trying to understand where it comes from. One of the factors in the biomarker findings, she added, is sleep quality; when exposed to E2, women who are poor sleepers are less responsive than good sleepers, which brings us back to the issue of stress.

Dr. Maki noted that precision medicine is the only route forward. Subgroups with different responses are well accepted for conditions such as premenstrual dysphoric disorder and post-partum depression. If there are different responses to estrogen sensitivity in mood and health disorders, why shouldn't that be true for cognitive aging? "This is not a separate category, it's still the same brain."

In closing, the participants agreed that considerably more preclinical and clinical research is needed on the various types of HT women are using, including careful consideration of timing relative to menopause onset, menopause etiology, age, and dosing. Understanding the interaction of estrogens and progestogens is important because each affects the other's receptors, and in many cases the effects are in opposition, Dr. Bimonte-Nelson pointed out. E2, several participants noted, is a "Goldilocks molecule." It must be just right for any particular woman, "but the question is, how do we actually know what those parameters are?" Dr. Roberts noted that the IUD appears to be making a comeback, many embedded with a progestin, with the women getting no estrogen, but not much seems to be known about what cognitive effects are likely as these women age. The effects of oral contraceptives and SERMS have also been little explored. Because the hormonal trigger for menopause s poorly understood (it may be driven by an LSH and FSH dynamic alone, for example), the researchers discussed that ovarian depletion is not the only valid model of menopause.

## 4. SESSION 2: EFFECTS OF HORMONE THERAPY ON BRAIN STRUCTURE AND FUNCTION

### 4.1 Hormone Therapy and Biomarkers of Cognitive Aging and Alzheimer's Disease

Kejal Kantarci, M.D., from the Mayo Clinic, described results from the KEEPS Neuroimaging Biomarkers Ancillary Study, conducted to investigate effects of hormone treatment on surrogate imaging biomarkers of cognitive health. Previous studies of estrogen's effects in ovariectomized rodents and rhesus monkeys showed increased synapse formation and connectivity in the brain (in the hippocampus

and prefrontal cortex), decreased β amyloid deposition, increased cerebral blood flow and metabolism, and interaction with APOE genotype. The researchers studied HT effects by looking at imaging biomarkers used to detect cognitive changes in the clinical and pathological progression of dementia.

The women in the Mayo Clinic study were a subset (118) of those in the KEEPS clinical trial, aged 42-58, all within three years months of last menses and with no previous cardiovascular events. (For HT formulations in KEEPS, see Gleason, above.) Menopausal HT was given for four years and imaging was done at intervals over a three-year period after the end of menopausal HT, for a total of seven years of follow-up in the participants after initiation of HT. They measured whole brain atrophy and ventricular expansion, the volume of white matter hyperintensities (as a surrogate for ischemic small vessel disease in the brain), and dorsolateral prefrontal cortex volume.

Results of the structural MRI study show that the short-term effects of estrogen treatment on the vascular system and on brain structures may be different from long-term effects. 1) Greater rates of increase in ventricular volumes were detected in recently postmenopausal women who received oral CEE therapy compared to placebo. But this was transient and was only present during the four-year treatment phase. Three years after menopausal HT was concluded, the trajectory of change in ventricular volumes was not different from placebo. 2) HT with transdermal estradiol preserved prefrontal cortex volumes more than for the placebo group over seven years. 3) Women who received HT with transdermal E2 for four years had lower levels of amyloid-β on PET imaging after seven years, particularly if they were APOE ε4 carriers, compared to placebo. 4) There was no effect on cognitive function over seven years.

In conclusion, Dr. Kantarci said, the results show that the effects of early postmenopausal HT on biomarkers of cognitive health differ by formulation and route of administration. Also, the effects are modulated by *APOE* ε4 status, which increases the risk for cognitive decline and Alzheimer's disease. The findings, she noted, have important implications for individualized approaches to prevent Alzheimer's disease in women, particularly for *APOE* ε4 carriers, who are the most vulnerable.

### 4.2 Estrogens, the Timing Hypothesis, and the Remediation of Cognitive Aging: Lessons from ELITE

Victor Henderson, M.D., Director of Stanford University's Alzheimer's Disease Research Center, described findings from a cognitive study conducted as part of ELITE, the Early versus Late Intervention Trial of Estradiol. The ELITE trial, led by Howard Hodis, was designed to test the "critical window" (timing) hypothesis, which posits that the effects of estrogen therapy depend upon the age of initiation or the timing on relation to menopause, with younger postmenopausal women benefiting the most. Some evidence suggests there may be a limited "window of opportunity" for beneficial effects of HT in women after menopause, so the timing question is of considerable interest to researchers in the field.

The primary endpoint in ELITE was subclinical atherosclerosis, while the endpoints of the ancillary cognitive study, ELITE-Cog, were composite measures of verbal memory (primary ELITE-Cog endpoint), executive functions, and global cognition. A total of 567 postmenopausal women participated in ELITE-Cog, in which Dr. Henderson and his colleagues examined whether oral E2 (1 mg/d) administered within six years of the initiation of menopause would affect cognitive functions differently than oral estradiol begun 10 or more years after menopause.

The results of ELITE-Cog show that E2 neither improves nor harms cognitive abilities, regardless of time since menopause. For younger postmenopausal women, the ELITE-Cog findings are consistent with results from KEEPS-Cog and WHIMSY and provide stronger evidence for longer-duration HT (oral E2 over five years) in the younger group of women. The ELITE-Cog findings in older postmenopausal women are largely consistent with results from WHIMS, WHISCA, and WHIMS-ECHO.

Overall, Dr. Henderson concluded, the findings from ELITE show there is neither meaningful cognitive benefit nor cost from HT when initiated by healthy postmenopausal women and used for up to five years, particularly in the midlife period when women are most likely to consider HT for other symptoms. He noted that the findings aren't generalizable to other HT formulations, and the findings from ELITE don't provide direct evidence about risk for Alzheimer's disease.

### 4.3. Nonhuman Primate Models of Hormone Therapy Effects on Cognitive Aging

Mark Baxter, Ph.D., of the Icahn School of Medicine at Mount Sinai, described studies in rhesus monkeys to investigate the effects of HT on cognitive aging. Rhesus monkeys are good models for studies of aging and HT, Dr. Baxter noted, because the animals have no natural Alzheimer's disease and have a menstrual cycle like that of humans (with a 20-year-old monkey equivalent to a 60-year-old human). Both monkeys and humans show age-related impairment of spatial working memory, dependent in monkeys on synaptic health in the prefrontal cortex—a region homologous in monkey and human brains. The monkeys showed differences as a function of aging and treatment on tests of spatiotemporal working memory, and for morphological changes in spine density of pyramidal neurons in area 46 of the prefrontal cortex.

The experiments were conducted in four groups of ovariectomized rhesus monkeys: two groups of young monkeys who received either E2 HT or vehicle, and two groups of aged female monkeys who also received either E2 HT or vehicle. Because some evidence suggests there may be a limited "window of opportunity" for beneficial effects of HT after menopause in women, additional groups of aged, ovariectomized monkeys were tested with a treatment protocol designed to determine effects of delayed treatment or withdrawn treatment. Delayed treatment addressed the hypothesis that E2 treatment initiated more than 2 years post-ovariectomy would have a reduced effect on cognitive function. Withdrawn treatment mirrored current clinical advice to women to use HT in the initial postmenopausal period, then discontinue it. Two periods of cognitive testing were accomplished to assess treatment effects on cognition over time in these groups.

The results of the experiments showed that: 1) Surgical menopause in aged monkeys produces a loss of thin dendritic spines on prefrontal pyramidal neurons, accompanied by a deficit in spatiotemporal working memory. Both changes can be remediated by cyclic E2 treatment; however, continuous E2 treatment, or a combination of E2 with progesterone, is ineffective. 2) Cyclic E2 remains effective in improving working memory in monkeys even if treatment begins more than two years after ovariectomy. 3) There is a very close relationship between measures of "synaptic health"—for prefrontal cortex, density of thin dendritic spines, and mitochondrial morphology in presynaptic terminals—and cognitive abilities dependent on the prefrontal cortex. This presents a target for developing therapeutics aimed at improving prefrontal cortex function in aging.

Aged, ovariectomized rhesus monkeys treated with vehicle who experienced a "double hit" from the effects of aging plus loss of estrogens had the worst outcome, resulting in a poor functional state in the prefrontal cortex, Dr. Baxter noted. Modification of synaptic structure, he concluded, has the potential to have a dramatic impact on cognitive aging because a state of good synaptic health inoculates against insults.

### 4.4 Hormone Therapy and Brain Function: What Role Do Hot Flashes Play?

Pauline Maki, Ph.D., Director of the Women's Mental Health Program at the University of Illinois–Chicago, began by noting that while hot flashes are the cardinal symptom of menopause, not much is actually known about what happens in the brains of women who experience them because studies are generally based on self-reported hot flashes, making the data unreliable. She described experiments that measured hot flashes objectively with ambulatory skin conductance monitors (which revealed that women under-report hot flashes by 40%). The studies using these physiologic measures contradicted previous

findings in showing that hot flashes are associated with memory declines and structural and functional brain abnormalities.

In the natural life cycle, verbal memory in women declines during the menopausal transition, can be increased with estrogen therapy, and may rebound postmenopausally; the critical window for memory is in perimenopause. Verbal memory is mediated by the hippocampus, and Dr. Maki's studies showed that menopausal women who started HT early in the transition had better memory and hippocampal function later in life. HT was associated with enhanced activation in the left hippocampus in particular. This may suggest that HT keeps the hippocampus "young," because other work demonstrates that left-right hippocampal activity and connectivity is heightened as estradiol levels increase – a change from the typical reliance on the left hippocampus in the premenopausal period. A preliminary study based on a larger study of cardiovascular and brain health showed that greater numbers of objective hot flash incidents, especially at night, are associated with greater disruption of the default mode network. To test the hypothesis that hot flashes themselves might lead to memory problems, the researchers designed a small randomized clinical trial to see if eliminating hot flashes would allow memory to bounce back. An anesthesia procedure called stellate ganglion blockade reduced moderate-to-severe hot flashes in the women by 50%. Memory recovered in direct relationship to the magnitude of the improvement in objective hot flashes. A larger study is underway, funded by the NIA.

Dr. Maki said that hot flashes can act independently of the hormonal changes associated with menopause to influence brain aging and lower brain reserve, making women more vulnerable to Alzheimer's disease. Results from WHIMS on the relationship between HT and Alzheimer's disease risk differ from those based o WHI mortality data, for reasons unknown, so the differences remain to be worked out. Overall, Dr. Maki concluded, there are still major gaps of knowledge related to HT effects on brain function in women with hot flashes; HT effects on brain function if treatment begins in perimenopause; and whether HT affects Alzheimer's disease risk if started early.

## 5. Panel Discussion

Dr. Maki's presentation elicited several questions about hot flashes. Hot flashes are not typically measured objectively in HT studies and their etiology is poorly understood. Dr. Maki explained that hot flashes are not strictly modulated by estrogens; hot flashes are also associated with a cortisol surge. Some women have been documented to have 35 surges a day. Basic science shows that the hippocampus loses resilience to the cortisol surge when estrogen levels drop, so it's "a perfect storm" for memory performance decline in menopausal women. The results of this work are probably not generalizable to women with mild to moderate hot flashes, Dr. Maki noted, but rather are seen among women with moderate to severe hot flashes, and in the general population about 30% of women meet that criteria (some with symptoms for more than 10 years after the final menstrual period). While not all women benefit from estrogen in the same way, the findings point to a subset of women who would benefit from estrogen-containing HT in the absence of contraindications.

In response to other questions, Dr. Maki said objective hot flashes show diurnal variation, in line with diurnal variation in core body temperature. There appears to be "something special" about nighttime hot flashes still not explained. Estrogen containing hormone therapies have been shown to have positive effects on cognitive function after surgically induced menopause, so using estrogens to treat sleep-related problems during the menopausal transition could have an additive effect. Exploring the complex interplay of hot flashes, sleep, and biological effects of estrogens—keeping the whole complex of both direct and indirect effects of estrogens in mind—would be a great direction for basic science, Dr. Maki said.

The panel shifted to discussion of imprecise definitions for subjective cognitive complaints in perimenopausal women. Subjective cognitive complaints do not always align well with objective impairment. What cognitive tests map onto complaints subjects report as "fuzzy brain?" Several

participants noted that despite limitations, they find neuropsychological assessments of cognition to be reliable in midlife women with cognitive complaints. What women report as memory problems may in fact be attributable to attention deficits. Dr. Maki said that a study by Neill Epperson's group measured inattention objectively with a rating scale, the women who met criteria for ADHD and were exposed to a stimulant showed improvement in cognitive tests and brain functioning.

There was agreement that one gap in our knowledge of the relationship between menopause and cognitive decline is how to map subjective reports of symptoms onto objective measurements. This is true especially in something like sleep, which is based heavily on self-reporting (although technologies such as actigraphy and other wearables are improving this). The bottom line is that there are many factors at play for which data are still lacking. In many cases the technology exists, but very large samples and data points are required. "We know there are associations," one participant observed, "but the question is whether or not we can measure them in randomized trials of HT."

The session ended with a brief discussion of sex differences relevant to research in the field. For example, what evidence is there, other than longevity, that women are disproportionately at risk for Alzheimer's disease? In some, but not all studies, women are more sensitive to APOE ε4 and experience the deleterious effects on cognition beginning much earlier, in midlife that may coincide with menopausal transition. At perimenopause, glucose metabolism declines, white matter degenerates, and β-amyloid deposition may begin, putting women on a path to longer-term cognitive decline. However, some studies have found greater risk for men, and sex differences have not been observed in AD biomarkers.

Dr. Einstein said gender differences are something she "thinks about all the time." Whether or not prevalence is higher in women, AD is a multifactorial disease, and it is likely that men and women have different risk factors. So, "it still behooves us to think about genders differences.' Further, the impact of risk factors on cognitive outcomes may vary between men and women.

## 6. SESSION 3: BIOLOGICAL RISK FACTORS FOR COGNITIVE AGING AND DEMENTIA

### 6.1 Sex-Specific and Polygenic Risk Factors

#### *6.1.1 The Effects of E2 Replacement on Memory and Brain Aging in Middle-aged Women with BSO: The Quandary of Women with BRCA1/2 Mutations*

Gillian Einstein, Ph.D., Chair of Women's Brain Health and Aging at the University of Toronto, opened this session by stressing the need to think of "menopause" not as monolithic but of many types, each with distinct hormonal changes and varied health and cognitive consequences. Some medical societies have classified menopause into four types for clinical purposes: spontaneous/normal (>50), early (40-45), premature (<40), and induced (through surgery or other causes such as chemotherapy). However, most research studies on menopause and cognition mix menopause types, age at onset, and causes. In an analytic review, Dr. Einstein and her co-authors found poor differentiation of menopausal types in 50 research papers on menopause and cognition published in 2016. Taking menopausal type into account is especially important in considering hormone replacement strategies considering evidence showing, for example, that early or induced menopause is associated with increased risk for cognitive impairment.

The estimated lifetime risk for Alzheimer's disease in women at age 45 is approximately 1 in 5 (twice the risk for men), and a central question is whether E2 loss/menopause contributes to the elevated risk in women. Many risk factors related to reduced estrogen come into play, Dr. Einstein noted, such as chemotherapy, adjuvant therapy, primary ovarian insufficiency (POI), other endocrine disorders, and oophorectomy. Her research focuses heavily on specialized types of menopause, such as younger women who have experienced induced menopause before age 50 and may have added risk factors such as

BRCA1/2 mutations. Findings have shown that women who undergo induced menopause prior to age 50 have higher incidence of Alzheimer's disease than women who keep their ovaries, and those with earlier induced menopause (at ages 38-43) have a steeper slope of cognitive decline, thus increasing the burden of Alzheimer's disease pathology. Imaging has revealed decreased amygdala volume and hippocampal cortical thickness among older women who underwent menopause early.

Women who undergo menopause prior to the age of spontaneous menopause are an important population to study. Epidemiological studies show that women with oophorectomy prior to age 50 are at higher risk for dementia and all causes of death. They have decreased verbal memory function if lost estrogen is not replaced within three months after oophorectomy. Many healthy younger women with BRCA1/2 have undergone BSO before the age of 50 as a prophylaxis. Dr. Einstein and her colleagues is looking at young women who undergo bilateral salpingo-oophorectomy (BSO; both ovaries and fallopian tubes are removed).

Einstein is studying behavior and brain changes in women within five years of BSO. Her team finds changes linked to Alzheimer's disease, including reduced working and spatial memory, and increased sleep disruption within five years after BSO, but that E2 therapy helps preserve spatial memory, frontal cortical areas, hippocampi, and sleep in women with BSO. However, those unable to take E2 are left in a quandary.

Finding ameliorative therapies for women with all types of menopause, Dr. Einstein said, will require looking at factors such as mitochondrial dysfunction, disturbed sleep, and increased inflammation. The picture is complex, and risks and treatments will vary by type. Studies of induced menopause, she added, could be potentially revealing about the earliest stages of incipient Alzheimer's disease, thus aiding efforts to better target changes and perhaps intervene.

*6.1.2. Sex Differences in the Molecular and Genetic Regulation of Memory: Implications for the Development of Sex-specific Treatments for Memory Dysfunction*

Karyn Frick, Ph.D., of the University of Wisconsin–Milwaukee, described research in mice to determine how E2 regulates memory (in both sexes); to understand why hormone loss leads to memory dysfunction in menopause and Alzheimer's disease; and to develop novel avenues for treatment. E2 improves memory in both females and males, Dr. Frick explained, but it does so via different signaling mechanisms. Studies involving a transgenic mouse model of Alzheimer's disease revealed sex and genotype effects that may shed light on the increased risk for Alzheimer's disease in women who are *APOE ε4* carriers, and why they have responded poorly to treatment with hormone therapy.

Studies in young adult ovariectomized mice showed that the ability of E2 to enhance object recognition and spatial memory consolidation depends on activation of numerous receptors, cell-signaling pathways, epigenetic processes, and genes, as well as dendritic spine remodeling. Findings indicate that phosphorylation of extracellular signal-regulated kinase (ERK) is necessary for E2 to increase dorsal hippocampal mTOR-mediated local protein synthesis, CA1 dendritic spine density, and memory consolidation. ERK and mTOR phosphorylation influences dendritic spine density in the medial prefrontal cortex (mPFC). Dr. Frick and her colleagues showed that the mPFC can mediate object recognition and spatial memory consolidation in ovariectomized mice, and the ability of hippocampally-infused E2 to enhance memory depends on simultaneous activation of the mPFC. Related studies in male mice (gonadally-intact or castrated) showed that E2 infusion into the dorsal hippocampus enhanced object recognition and spatial memory consolidation to a similar extent as in females, but the signaling mechanisms differ. The memory-enhancing effects of E2 in females depend on ERK activation but are independent of ERK in males and may depend instead on CREB phosphorylation.

Other studies are investigating the interrelationships between sex, *APOE* genotype, and E2 in the risk of dementia in Alzheimer's disease. The research is being done in an EFAD transgenic mouse model of Alzheimer's disease (expressing human APOE ε3 or APOE ε4). Researchers have found that male ε3 mice have better spatial and object recognition memory than female ε3 or ε4 mice of either sex. APOE ε3 males also have the lowest ERα levels in the dorsal hippocampus relative to all groups, while ε4 females have the lowest pCREB, synaptophysin, and PSD-95 levels. Among EFAD females, E2 enhances memory consolidation in E ε3, but not ε4, females. These data mirror APOE-genotype effects in women treated with hormone therapy.

The researchers found that a novel and specific ERβ agonist, EGX358, enhances object recognition and spatial memory consolidation in young adult ovariectomized mice via three routes of administration. Ongoing studies are investigating effects in models of hot flashes, anxiety, and depression.

*6.1.3 Sex-Specific Molecular Drivers of Alzheimer's Disease Risk and Resilience*

Tim Hohman, Ph.D., of Vanderbilt University Medical Center, described research that reveals fundamental sex differences in the pathology of Alzheimer's disease. The studies are part of efforts to discover pathways of resilience that can inform therapeutic interventions. Precision medicine is usually discussed as providing the right drug for the right patient at the right time, but Dr. Hohman said it is also about the "power of individual stories" to drive discoveries and therapies that can benefit the larger population. He offered, as examples, an HIV-resistant adult male and a rare genetic variant found in a tiny number of Dallas Heart Study patients that led to now widely used drugs for AIDS and heart disease prevention.

Similarly, Dr. Hohman and his colleagues are now looking for sex-specific genetic predictors of neuropathology in Alzheimer's disease to get at novel treatments. Amyloid build-up starts 15-20 years before onset of the disease, and there are genes all along the "amyloid cascade" that play a role in various ways. There may be shared genetic risk factors for each of the processes, and sex-specific factors that contribute to neurodegeneration, but this not been examined comprehensively.

Dr. Hohman summarized findings from various studies that have confirmed fundamental sex differences in the neuropathology of Alzheimer's disease, not just in occurrence but also in cognition. Females show more longitudinal hippocampal atrophy and cognitive decline. *APOE* e4 association with Alzheimer's disease is stronger in females (though there appears to be an inversion later, when men become more at risk). The researchers have been looking for possible sex-specific differences in the genetic architecture of Alzheimer's disease by looking first at APOE and then moving to the larger genome. The whole-genome analysis, Dr. Hohman said, led to greater specificity in what genes and pathways to look for.

Among the findings so far: 1) Compared to males, females show a stronger association between *APOE*-ε4 genotype and cerebrospinal fluid levels of tau. 2) Similarly, when compared to cognitively normal males, cognitively normal females show a stronger association between baseline amyloid levels (measured with PET) and entorhinal cortex levels of tau measured with PET. 3) Females also show a stronger association between baseline CSF amyloid levels and longitudinal accumulation of tau compared to males. Specifically, at higher levels of baseline amyloidosis (lower CSF Aβ42), females accumulate tau more rapidly than males. 4) The genetic predictors of amyloid and tau differ by sex. (PET and clinical AD results are pending.) 5) Genetic predictors of resilience to amyloid and tau also vary by sex (with large-scale discovery work on this topic also pending).

*6.1.4 Predicting Cognitive Aging and Decline from Pre-treatment Neuroimaging*

Shelli Kesler, Ph.D., of the University of Texas at Austin, described results from chemotherapy-related research that show how neuroimaging can be used to identify risk and resilience factors early on, as

predictors of cognitive outcomes. The researchers were also able to identify neurophysiologic subtypes of heterogeneous cognitive impairments.

Chemotherapy is a known risk factor for cognitive impairment; even small quantities can cause lasting harm to neuronal structure and functions. About 30-60% of patients experience cognitive impairment after chemotherapy, but not knowing who will become impaired makes it hard to manage clinical treatment. Typical factors like age, education, and mental health aren't reliable predictors, Dr. Kesler noted. Kesler and her team used fMRI to measure functional connectivity in the brain, to identify factors associated with cognitive impairment risk and resilience.

The women in the study were newly diagnosed with breast cancer but had not yet undergone any treatment; they were around 50 years old, highly educated, 31% postmenopausal, and primarily with early-stage disease. The researchers wanted to see if properties in the brain's organizational network (the "connectome") of this pre-treatment group could predict who would and would not have cognitive impairment a year after chemotherapy treatment. Three machine-learning models were evaluated based on various combinations of patient medical variables and properties in the connectome. Cognitive status was measured using five neuropsychological tests. (Cognitive impairment was defined conservatively, corresponding clinically to mild to moderate impairment.) One of the three models proved 100% accurate, so the study of 74 patients will be replicated in 300 patients. Another model predicting self-reported patient outcomes also did very well. Menopausal status and history of hormone blockade treatment were not significant predictors in any of the models, but this may reflect the need for more precise measurement of these variables such as menopause prior to age 50, cause of menopause, and/or estrogen levels.

Given that cognitive impairment is not likely dichotomous, Dr. Kesler and her team employed machine learning methods further to identify neurophysiologic subtypes of cancer-related cognitive impairment. Retrospective fMRI data from 80 chemotherapy-treated breast cancer survivors and 103 healthy female controls identified three separate subgroups, including one at very high risk for cognitive impairment. Closer examination identified multiple risk factors that weren't captured in the broader group, such as minority status, shorter time off chemotherapy, and heavy exposure to stress. Surprisingly, Dr. Kesler noted, the highest-functioning group also had the greatest severity of disease and incidence of locoregional radiation therapy. These findings contradict results from previous studies but highlight the importance of examining subtype-specific risk/resilience factors. For example, this high functioning, high disease severity group seems to be characterized by some type of resilience that might be explained by estrogen or other factors that weren't included in the data.

## 6.2 Metabolic and Epigenetic Factors

### *6.2.1 Bioenergetics of Menopausal Transition and Cognition, and Challenges to Achieving Precision Hormone Therapy*

Roberta Brinton, Ph.D., Director of the Center for Innovation in Brain Science at the University of Arizona College of Medicine, opened by emphasizing estrogen's role as a major "systems of biology regulator" in the brain, which means estrogen deprivation can affect multiple systems and functions. While women are experiencing wide variability of endocrine and neurological functions during the menopausal transition (80% of women are symptomatic), glucose metabolism in the brain is also declining. Brain metabolism is of major importance because the brain has high energy demands, and deficits can impair critical functions such as encoding new information learning, retrieval and memory, and neural network generation and repair. Brain glucose hypometabolism is an early hallmark of Alzheimer's disease, and is seen in the high-risk APOE ε4 population and in familial AD.

Among its roles, estrogen regulates glucose uptake, helping the brain generate the ATP energy it requires. A decline in glucose metabolism related to decline in brain estrogens activates a "starvation response" in which the brain shifts to utilize ketone bodies as an auxiliary fuel. Dr. Brinton described a pathway that explains how the starving brain, when normal estrogen action is disrupted, could utilize myelin as a source of ketone bodies to fuel ATP production. Electron micrographs show loss of myelin integrity coincident with perimenopause. Consistent with preclinical discovery findings, MRI structural imaging in peri, menopausal and postmenopausal women indicate a significant decline in white matter volume and a modest decline in select gray matter regions. Further, β-amyloid deposits can appear early in the endocrine-aging transition in female brains. In new work, the researchers are investigating activation of the immune system during the perimenopausal transition affects the immune system. Most recent analyses detected transcripts unique to T-lymphocytes in brain which is being further investigated in post-mortem brain. These findings are relevant to the greater incidence of autoimmune diseases in women during the perimenopausal period.

In the over-65 population, 11% of people are predicted to get Alzheimer's disease. Metabolic profile offers one possible biomarker to help predict risk in still-healthy women. In a study of 502 healthy women, the researchers found those who were metabolically most healthy had the best cognition, while those at risk for metabolic syndrome had the greatest cognitive decline. The "drivers" in the latter group were women with APOE *ε4* (which was distributed equally in the groups). Though sex or APOE genotype can't be changed, it is possible, Dr. Brinton noted, to change metabolic health and potentially reverse the risk of AD.

Turning to precision medicine in HT, particularly in relation to "windows of opportunity" for therapeutic interventions to reduce the risk of neurogenerative diseases, she said aging must be viewed not as a linear process, but a series of transitional states that are unstable and potentially reversible. Complex systems have critical thresholds, and the "tipping points," like stress or sleep deprivation, that will vary for different people. Interventions such as HT are likely to be most effective when symptoms first appear during the perimenopause or early menopause. In addition to mitigating common symptoms such as hot flashes, HT is likely to have therapeutic benefit for complex cognitive functions over simple tasks.

*6.2.2 Metabolic Correlates of Accelerated Cognitive Aging in Women at Genetic Risk for AD*

Natalie Rasgon, M.D., Ph.D., Director of Stanford University's Center for Neuroscience in Women's Health, reviewed studies of moderators and mediators of cognitive aging across the human lifespan to discover windows of vulnerability that place women at a greater risk of accelerated cognitive aging. Estrogens are among the most powerful mediators of hippocampal structure and function, but not the only ones; BDNF, progesterone metabolites, and cortisol are among the many other mediators affecting neuroplasticity in brain regions implicated in early cognitive decline. The researchers are looking, for one thing, at the impact of both peripheral and central insulin resistance on neuroplasticity. Understanding independent and iterative mechanisms of insulin resistance in the context of estrogen effects is highly salient because both are modifiable mediators of cognitive aging, whereas sex and genetics are non-modifiable moderators. Furthermore, the importance of identifying early risk factors for accelerated cognitive decline lies in protection from developing end-stage endophenotypes. For example, while diabetes is not curable but is a treatable, and insulin resistance and pre-diabetes are potentially reversible.

Dr. Rasgon said studies of the neurobiology of stress and resultant psychopathology have shown that exposure to early-life adversity has long lasting effects on brain function. Childhood trauma can moderate effects of insulin resistance in the brain in a sex-specific manner. She and her colleagues found that, of all subtypes of childhood trauma, emotional abuse (as measured by the CTQ, or Childhood Trauma Questionnaire) emerged as a consistent predictor of neuroendocrine correlates of cognitive aging.

In studies of estrogen therapy's effects in the brain, the researchers found that in a group of middle-aged, postmenopausal women at genetic risk for Alzheimer's disease, longer endogenous estrogen exposure, as well as exogenous exposure to estradiol, was associated with positive effects on regional cerebral metabolism, whereas exposure to conjugated estrogens was associated with the metabolic decline in mesolimbic region.

The researchers also looked at telomere length as a cumulative biomarker of oxidative stress and allostatic load and found that duration of endogenous estrogen exposure also correlates positively with telomere length. Estrogen-telomere length data are intriguing not only in relation to brain function in menopause, but also in a context of early programming of stress vulnerability. As such, Epel et al. reported familial transmission of shortened telomeres from mothers with a history of stress to their newborns on the first day of birth. Finally, the well-known genetic risk factor for late-onset AD, APOE *ε4*, moderates the correlation between telomere maintenance and hippocampal volume in HT users. Studies of HT and telomere length in APOE *ε4* carriers and non-carriers found that HT (subjects were taking either estradiol or CEE alone or estrogen+progesterone) might decelerate cell aging in APOE *ε4* carriers, but not for non-carriers. Though sex and APOE status are not modifiable, Dr. Rasgon said, improving or resolving insulin resistance could potentially preserve telomere length. In studies to explore mechanisms of action, she noted, the metabolite acetyl carnitine is emerging as a modulator of insulin resistance as well as a mitochondrial epigenetic modulator of glutamatergic function, with corresponding brain plasticity in cortico-hippocampal circuits.

Taken together impacts of multiple events—being female, early-life trauma, developing insulin resistance, with corresponding deficits in other regulatory networks (glutamatergic function, oxidative stress etc.)—predisposes some women to accelerated cognitive aging and by the same token, might help in identifying groups of women uniquely sensitive to estrogen treatment. Dr. Rasgon and her colleagues are now conducting a longitudinal study modeling proposed modulators and mediators of accelerated cognitive aging in women over a 25-year span. The patterns that emerge may be a useful step toward personalized medicine.

## 7. Panel Discussion

The previous presentations raised questions about resilience and its influence in menopause and aging. The group discussed plausible factors that could explain and promote resilience. Animal studies have shown that moderate stress early in life can lead to higher resilience, but more severe stress is negative and could be a risk factor, raising the issue of whether there is a threshold of vulnerability. Dr. Rasgon emphasized that emotional abuse measured in previous studies relates only to the occurrence in childhood (before puberty), not across the lifespan. Her own research has indicated that childhood emotional abuse, by far, has the biggest impact, and that women in midlife tend to underreport childhood abuse and trauma on the Childhood Trauma Questionnaire (CTQ). She noted that studies have shown a relationship between childhood trauma and early age of menopause. Is the issue of childhood trauma tied at all to the issue of gender, and not just sex, one participant questioned; that is, do ideas of masculinity and femininity come into play in reports of childhood trauma? Dr. Rasgon said her current studies have been done only in women, but other research has found childhood emotional abuse to also affect men, though the association is stronger in women for glutamatergic effect.

Participants moved to a discussion of telomere length and how well it might serve as an indicator of vulnerability, therapeutic intervention, and resilience. Telomere length, it was suggested, considered together with estrogen, metabolic profiling, and other factors, might lead to better and deeper phenotypes that help suggest who would benefit from therapeutic intervention.

The discussion included further consideration of the lingering question of how long to treat with HT, considering what is known so far. Estrogen therapies affect metabolism, so withdrawing estrogen therapy

later in life only delays the metabolic changes. Past recommendations of five years for HT were based heavily on breast cancer risk and earlier hormone formulations, not those currently used. Dr. Brinton urged going back to the "billions of data points" available, back into electronic medical records, to see what it reveals about HT and its risks and health benefits. Then, she said, researchers should go into the lab and do experiments, in a sort of reverse translation, instead of starting at the discovery, cellular level, or animal model stage.

The question was raised as to whether metabolic differences between men and women might inform useful dietary interventions. In other words, "do women do a ketone diet and men the Atkins?" So far, little research has been done to investigate what the right nutritional interventions might be in sex-specific phenotypes relevant to various diseases.

## 8. Knowledge Gaps and Research Recommendations

The workshop ended in a roundtable discussion with initial mention of the growth in the field in the last few decades, and then focused on how this expanding rich framework of data inform knowledge gaps and recommendations for moving the field forward; what do we know, and where does that take us next? The "field" in question, it was agreed, focuses on cognitive functioning in women who are affected by all types of menopause. The group proposed the following research directions and questions as areas of priority. It is important to stress that a single approach to HT will not be appropriate for all women. Indeed, studies in animal models and humans suggest that HT may be beneficial, however, putative interactions between menopause variants, hormone types, including whether estrogens are unopposed or opposed by progestogens included in studies still obscures the ability to systematically determine which parameters impact outcomes in studies collapsing across these factors. This, in turn, can attenuate clear conclusions, driving discovery of clinical therapeutic applications.

### 8.1 Therapies

Fundamental gaps remain in our knowledge of HT in relation to formulation, dose, and duration for cognitive and brain aging. Among the questions that remain unresolved:

- Do cognitive effects of cyclic estrogens and cyclic progestogens differ from the continuous formulations used in major clinical trials of menopausal HT?
- How early should HT be initiated during the menopausal transition?
- Are HT interventions most efficacious when given during the early and/or late perimenopausal stage?
- When should HT interventions end during post-menopause to confer maximal benefits and also minimize harm?

One major goal in the field is to identify agents that effectively treat menopausal symptoms but do not raise safety signals as is the case for some current commonly utilized agents. Better understanding of the effects of other estrogenic compounds is needed. Women—and men—are exposed to other estrogenic and antiestrogenic compounds, including phytoestrogens, other xenoestrogens, and various types of selective estrogen receptor modulators (SERMS). What are the short- and long-term cognitive consequence of, for example, soy isoflavones, tamoxifen, leuprolide, raloxifene, and phthalates?

### 8.2 Precision medicine

Informed recommendations are needed for prescribing HT that consider individual differences in response, such as an individual's age, genetic background, reproductive history, and other factors. There was agreement about the need for a broader life course perspective to better understand how early life exposures influence reserve and resilience, which may modulate the response to menopause and HT. There is a need for more information from about the 30-55-year age range to cover before and through

perimenopause and early post-menopause. Other considerations in precision HT include biomarkers of therapeutic target and indicators of efficacy, therapy types (mono- versus combination), routes of administration, and duration of exposure.

Precision medicine requires better knowledge of vulnerable populations and individual differences that affect treatment choices and outcomes. There are gaps of knowledge in the field for various populations of women, and researchers need to understand HT effects in large populations in order to study individual variations.

Knowledge is needed to answer questions such as:

- What groups of women may be at cognitive risk as a result of menopause or postmenopausal HT; for example, are women with diabetes, hypertension, or severe hot flashes at greater risk than those who are negative for these factors?
- Are women with premature and early menopause cognitively vulnerable in a way that other women are not? If so, what are the determining factors; e.g., altered lifetime exposures to endogenous estrogens, progesterone, and/or androgens?
- How do key biological, lifestyle, and/or environmental factors contribute to or reduce risk of cognitive decline and dementia in middle age and beyond?
- How do individual differences in resilience affect age-related cognitive decline and dementia?

One recommended priority is studies of the effects of chemotherapy-induced menopause and hormone blockade therapy in women with breast cancer, which has received very little attention in the literature. One in 8 women are diagnosed with breast cancer during their lifetime; thus, this is a very large group of women at risk for hormone-related cognitive effects and this domain of research is of high clinical significance.

**8.3 Biology and mechanisms**

Basic research is needed to identify how sex steroid hormones regulate cognitive function at the molecular, cellular, and brain circuit level throughout the lifespan. This information would provide important insights that could lead to better implementation of existing HTs and the development of novel HTs. Moving the field forward requires deeper knowledge of the biological processes involved in HT, aging, and cognitive decline. Research in systems biology is needed to understand transitions in menopausal aging, symptoms across the transition, and mechanisms of underlying symptoms.

Among the key questions to address in this area:

- How does HT affect dementia risk when used during the menopausal transition and early post-menopause, since this is the time during which women are most likely to consider menopausal HT for other symptoms? How do events that alter endogenous hormone levels—such as contraceptive use, pregnancy, anti-estrogen/aromatase inhibitor use, and surgical menopause—influence cognitive trajectory during aging?
- What factors influence individual differences in the response to HT? For example, does HT have different impacts on the brain and cognition in models of healthy aging vs. in models of diabetes, hypertension, etc.?
- What are the mechanisms by which females undergoing early surgical menopause benefit from HT, and are the mechanisms the same or different from effects of HT following natural menopause?
- What changes in the aging brain reduce positive responsiveness to hormone treatment? Are there changes that occur between perimenopause and post-menopause that render HT detrimental for women in their mid-60's or later, and can those changes be modified? Do different types of HTs have varied cognitive impacts depending on age at initiation?

- How do sex steroid hormones regulate cognitive function at the molecular, cellular, and brain circuit level throughout the adult lifespan? Such information would provide important insights that could lead to better implementation of existing HTs and the development of novel HTs.
- What explains the adverse signals that HT may accelerate brain atrophy in both younger (KEEPS) and older (WHIMS) women?
- Why does initiation/re-initiation of HT have different consequences for brain health depending on women's underlying health and menopausal status?
- How do key biological (e.g., genetic, hormonal), lifestyle (diet, exercise, education/cognitive stimulation, etc.), and/or environmental (stress, environmental estrogens) factors contribute to or reduce risk of cognitive decline and dementia in middle age and beyond?

Deeper molecular data in human cohort studies are needed, one workshop participant suggested. Currently, there are no strong biomarkers of whether response to any kind of HT will be positive or negative, nor are the underlying neuropathological changes understood that contribute to vulnerability or resilience to different types of HT.

### 8.4 Model systems and translational links

Preclinical models allow for direct testing of mechanisms. One issue to address is why many animal studies (rats, nonhuman primates) continue to suggest that menopausal HT *should* show cognitive benefit, while large, well-designed clinical trials consistently fail to demonstrate cognitive benefit. Better insight about this apparent paradox could inform questions to be asked in future clinical trials.

It was noted that model systems are needed that better reflect the etiology of menopause, aging, and Alzheimer's disease. Also, model systems should be strengthened to incorporate heterogeneity and genetic diversity that more accurately reflects human populations.

One workshop participant raised the need for more and better translational models of human disease (validity of model systems, stage of transition, intervention formulations relevant to humans). There is, for example, no science or quality control on HTs prepared by compounding pharmacies.

Another participant urged efforts to increase translational links in terms of cognitive processes that are particularly affected during the menopausal transition and after menopause; "brain fog" that may be related to attentional processes, for example, and which may not be detected by standard batteries of cognitive tests in both animals and humans.

### 8.5 Data and information exchange

The workshop recommendations include greater harmonization of data across clinical trials and cohort studies, as larger datasets are needed to study the number of variables necessary to draw inferences about precision HT as well as to identify molecular modifiers of treatment effects.

Large-scale electronic medical record data, it was noted, could be leveraged to investigate factors that modulate menopause associations with late-life cognitive decline or dementia. Although the depth of data in medical records is much lower, the breadth of data provides a rich opportunity to investigate genetic and phenotypic factors that interact with HT.

## 9. Overarching conclusion

The workshop concluded, noting that the cognitive aging, menopause, and HT independent and interactive research areas have grown in breadth and depth across the last few decades. This expansion is marked by a rich array of systematic, well-designed preclinical and clinical research studies which have

yielded important discoveries about the impact of menopause and HT on the brain and cognition. The need for personalized medicine to afford effective therapies highlights the many factors playing into HT optimization. It therefore follows that there is a critical need to acknowledge and test independent and interactive variables likely affecting outcomes of HT on cognitive aging. There was consensus that there should be increased discussion and communication across research disciplines, including crosstalk amongst scientists testing different species. The overarching conclusion was that these actions will enrich translation and discovery of therapeutic courses of action to optimize women's health across the lifespan.