

Is Timing Everything? New Insights into Why the Effect of Estrogen Therapy on Memory Might be Age Dependent

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Despite controversy concerning relative risks and benefits, hormone therapy (HT) remains the most effective treatment for vasomotor symptoms associated with menopause (1). Understanding the factors that determine the health outcomes associated with HT is therefore a key priority. One critical factor is the age at which treatment is initiated. Perhaps the most compelling evidence in support of that view comes from the very study that raised concerns about the risks of HT, the Women's Health Initiative (WHI). After an average of 7.1 years of follow-up, the WHI Estrogen-Alone Trial was stopped early because of an increased risk of stroke that was equivalent to an absolute excess risk of 12 additional strokes per 10 000 person years (2). After a mean of 10.7 years of follow-up in that trial, the impact of conjugated equine estrogens (CEE) on health outcomes, including coronary heart disease, myocardial infarction, colorectal cancer, total mortality, and a global index of chronic disease, was more favorable for women aged 50–59 at treatment initiation compared with women who initiated treatment at older ages (3). Within the 50- to 59-year-old group, the risk of coronary heart disease and total myocardial infarction was significantly reduced with CEE (3). (The risk of stroke was no longer elevated during the postintervention follow-up period). Thus early age at initiation was a critical determinant of the long-term impact of estrogen treatment. Such data support the hypothesis that estrogen treatment may confer benefits when initiated early, a hypothesis that is referred to in the literature as the “timing hypothesis,” the “window of opportunity hypothesis,” or the “critical window hypothesis.”

The timing hypothesis has been proposed to explain the impact of HT on memory and risk for Alzheimer's disease (4). Meta-analyses of observational studies revealed a significant 29% decreased risk of Alzheimer's disease in women who used HT (5). In contrast, the Women's Health Initiative Memory Study (WHIMS) revealed a doubling of the risk of all-cause dementia in older women (mean age 72 years) randomized to receive CEE plus medroxyprogesterone acetate (CEE/MPA) and no increased or decreased risk of all-cause dementia in women randomized to receive CEE alone (6, 7). The discrepancy between the observational studies and WHIMS has been attributed to differences in the timing of treatment initiation, with younger ages in women in the observational studies compared with the older women in WHIMS.

To date, 3 of 3 observational cohort studies that specifically examined the timing of HT initiation on the risk for Alzheimer's disease have provided support for the timing hypothesis (8–10). An ancillary study, the WHIMS of Younger Women, found no impact of either CEE or CEE/MPA on a measure of global cognitive function in women who were randomized to HT between the ages of 50 and 55 and who completed the assessment of global cognitive function on average 7.2 years after the trials ended, suggesting that treatments may be safer for younger women (11). It is unknown what percentage of women in the CEE-alone arm of WHIMS of Younger Women might have initiated CEE treatment during the critical window, because the average number of years since final menstrual period in the CEE group at the time of randomization was 9 years, and about 40% of women in that study arm had bilateral oophorec-

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Abbreviations: BDNF, brain-derived neurotrophic factor; CEE, conjugated equine estrogen; E₂, 17β-estradiol; HT, hormone therapy; MPA, medroxyprogesterone acetate; SIRT1, sirtuin 1; WHI, Women's Health Initiative; WHIMS, Women's Health Initiative Memory Study.

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tomy (12). Neuroimaging studies in humans indicate that verbal memory and hippocampal function are enhanced with early initiation of HT (13). Such human data are consistent with evidence from animal studies that the impact of 17 β -estradiol (E₂) on hippocampal function varies with age and is enhanced with early initiation (14–16).

In this issue of *Endocrinology*, Rao and colleagues (17) provide new insights into mechanisms that might explain why the impact of E₂ on cognition and brain function might depend on age at E₂ treatment. They focused on microRNAs (miRNAs), which are small noncoding RNAs that regulate gene expression by binding to complementary sequences in the 3'-untranslated region of target mRNAs, usually resulting in their silencing. A subset of miRNAs are known to mediate estrogen-regulated activities, and/or are regulated by estradiol (18). The hypothesis was that E₂ might differentially regulate miRNA expression in the ventral hippocampus of female rats, leading to altered gene expression important for neuronal function. The ventral hippocampus was chosen as the primary brain region of interest because of its critical role in memory function. To test this hypothesis, they treated ovariectomized 3-month-old (young adult) and 18-month-old (late middle age) female rats with sc E₂ (2.5 μ g) or vehicle control for 3 days. The E₂ dose resulted in physiological levels of E₂ levels that are comparable to levels observed during late diestrus/early proestrus, a phase of the rat estrous cycle shortly prior to the preovulatory increase in estradiol. The main question was how E₂ treatment, age, and the interaction of E₂ and age influenced miRNA expression.

In the first step of identifying candidate miRNAs in the left ventral hippocampus, the investigators used a microarray platform to probe 723 miRNAs and identify those with moderate-to-very high expression levels and high signal intensity. Of the 723 miRNAs, 34 were significantly altered by E₂ treatment regardless of age, 21 were significantly altered by age regardless of treatment, and 9 were altered by a combination of the 2 factors. In a second validation step, real-time quantitative PCR of RNA taken from the right ventral hippocampus confirmed that 7 of those 9 miRNAs were altered. Specifically, E₂ treatment significantly impacted 6 of the 7 miRNAs tested, age affected 2 of the 7, and the 2 factors combined affected 2 of the 7. Target prediction programs were then used to understand the physiologic impact of the E₂-regulated miRNAs. Based on those predictions, levels of sirtuin 1 (SIRT1), glucocorticoid receptor, γ -aminobutyric acid receptor A1, and brain-derived neurotrophic factor (BDNF) mRNA expression were measured and compared across treatment groups. Because degradation of mRNA transcripts is one mechanism whereby miRNAs decrease the protein expression of their target genes, it was expected

that miRNA expression would relate inversely to target mRNA expression. Indeed, that was the case for SIRT1, which was down-regulated in young, E₂-treated animals. SIRT1 expression has been linked to memory and neuronal plasticity (19), as well as anxiety through effects on monoamine oxidase (20). There was also a strong trend for an E₂-mediated increase in BDNF in the young animals. Notably, E₂ enhances hippocampal expression of BDNF, a growth factor that increases dendritic spines and enhances memory function (21, 22). Overall, these results indicate that E₂ is a critical regulator of miRNA expression in the ventral hippocampus generally, affecting gene targets that influence hippocampal function and memory.

Lastly, to determine whether these effects were specific to the ventral hippocampus, the authors examined miRNA expression levels in 3 other brain regions that are functionally connected to the ventral hippocampus: dorsal hippocampus, central amygdala, and paraventricular nucleus. Results demonstrated that magnitude of E₂ action on miRNA expression differed by brain region, with most brain regions having unique miRNAs that were significantly affected by both age and treatment. The exception was that E₂ altered expression of miR-7a, which targets SIRT1, in multiple brain regions. Notably, treatment with SIRT1-activating compounds has been shown to delay neurodegeneration in a transgenic mouse model and is thought to be a key mechanism involved in the beneficial effects of caloric restriction on memory and neuronal function (23) as well as the neuroprotective effects of resveratrol (24).

The demonstration that E₂ alters miRNA expression in an age-dependent manner opens up several avenues for future studies. First, as noted by the authors, a 3-day treatment exposure was sufficient to demonstrate proof of concept that E₂ can impact miRNA expression, but longer-term treatment is needed to better model how E₂ is used in clinical practice. Second, whether early and sustained E₂ treatment influences miRNA expression long after treatment is discontinued is an important area of future inquiry. Women who have an oophorectomy before the normal age at menopause show an increased risk for cognitive impairment or dementia later in life unless they are treated with estrogen until the normal age at menopause (25). Third, the study provides justification for investigating SIRT1 as a potential mediator of the impact of E₂ on memory and hippocampal function. SIRT1 has been implicated in the disruption of mitochondrial bioenergetics in Alzheimer's disease and mild cognitive impairment (26). Disruption of mitochondrial bioenergetics, in turn, has been implicated as a potential mechanism explaining the negative impact of estrogen on cognition when initiated in older women (27). Fourth, the increase in dementia observed with CEE/MPA rather than CEE alone suggests potential deleterious effects of MPA on brain function in older women (6, 7).

Understanding how MPA and other progestins might alter the impact of E₂ on miRNA levels can help to guide future clinical trials to identify estrogen/progestin combinations that might confer cognitive benefits. Lastly, in light of evidence that estrogen effects on cardiovascular disease and mortality are age dependent (3), it will be important to expand the evaluation of miRNAs in tissues other than brain.

In conclusion, there is compelling evidence that the impact of estrogen treatment on health outcomes varies with age and that health benefits may be most evident with early treatment. The precise mechanisms underlying such effects remain poorly understood. However, findings on miRNA expression presented by Rao and colleagues (17) provide new evidence that age-dependent effects on memory may be due to E₂ modulation of neuronal target genes that alter gene expression and influence hippocampal function.

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References

- de Villiers TJ, Gass ML, Haines CJ, et al. Global Consensus Statement on menopausal hormone therapy. *Maturitas*. 2013;74(4):391–392.
- Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA*. 2004;291(14):1701–1712.
- LaCroix AZ, Chlebowski RT, Manson JE, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. *JAMA*. 2011;305(13):1305–1314.
- Maki PM. Critical window hypothesis of hormone therapy and cognition: a scientific update on clinical studies. *Menopause*. 2013;20(6):695–709.
- Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. *JAMA*. 1998;279(9):688–695.
- Shumaker SA, Legault C, Kuller L, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. *JAMA*. 2004;291:2947–2958.
- Shumaker SA, Legault C, Rapp SR, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: The Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA*. 2003;289:2651–2662.
- Henderson VW, Benke KS, Green RC, Cupples LA, Farrer LA. Postmenopausal hormone therapy and Alzheimer's disease risk: interaction with age. *J Neurol Neurosurg Psychiatry*. 2005;76(1):103–105.
- Whitmer RA, Quesenberry CP, Zhou J, Yaffe K. Timing of hormone therapy and dementia: the critical window theory revisited. *Ann Neurol*. 2011;69(1):163–169.
- Shao H, Breitner JC, Whitmer RA, et al. Hormone therapy and Alzheimer disease dementia: new findings from the Cache County Study. *Neurology*. 2012;79(18):1846–1852.
- Espeland MA, Shumaker SA, Leng I, et al. Long-term effects on cognitive function of postmenopausal hormone therapy prescribed to women aged 50 to 55 years [published online June 24, 2013]. *JAMA Intern Med*. doi:10.1001/jamainternmed.2013.7727.
- Vaughan L, Espeland MA, Snively B, et al. The rationale, design, and baseline characteristics of the Women's Health Initiative Memory Study of Younger Women (WHIMS-Y). *Brain Res*. 2013;1514:3–11.
- Maki PM, Dennerstein L, Clark M, et al. Perimenopausal use of hormone therapy is associated with enhanced memory and hippocampal function later in life. *Brain Res*. 2011;1379:232–243.
- Silva I, Mello LE, Freymüller E, Haidar MA, Baracat EC. Onset of estrogen replacement has a critical effect on synaptic density of CA1 hippocampus in ovariectomized adult rats. *Menopause*. 2003;10(5):406–411.
- Markowska AL, Savonenko AV. Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. *J Neurosci*. 2002;22(24):10985–10995.
- Savonenko AV, Markowska AL. The cognitive effects of ovariectomy and estrogen replacement are modulated by aging. *Neuroscience*. 2003;119(3):821–830.
- Rao YS, Mott NN, Wang Y, Chung WC, Pak TR. MicroRNAs in the aging female brain: a putative mechanism for age-specific estrogen effects. *Endocrinology*. 2013; 154(8):2795–2806.
- Klinge CM. Estrogen regulation of microRNA expression. *Curr Genomics*. 2009;10(3):169–183.
- Gao J, Wang WY, Mao YW, et al. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature*. 2010;466(7310):1105–1109.
- Libert S, Pointer K, Bell EL, et al. SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive. *Cell*. 2011;147(7):1459–1472.
- Singh M, Meyer EM, Simpkins JW. The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology*. 1995;136:2320–2324.
- Luine V, Frankfurt M. Interactions between estradiol, BDNF and dendritic spines in promoting memory. *Neuroscience*. 2013;239:34–45.
- Gräff J, Kahn M, Samiei A, et al. A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration. *J Neurosci*. 2013;33(21):8951–8960.
- Pallàs M, Casadesús G, Smith MA, et al. Resveratrol and neurodegenerative diseases: activation of SIRT1 as the potential pathway towards neuroprotection. *Curr Neurovasc Res*. 2009;6(1):70–81.
- Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology*. 2007;69(11):1074–1083.
- Silva DF, Selfridge JE, Lu J, et al. Bioenergetic flux, mitochondrial mass and mitochondrial morphology dynamics in AD and MCI cybrid cell lines [published online June 4, 2013]. *Hum Mol Genet*. doi:10.1093/hmg/ddt247.
- Yao J, Irwin R, Chen S, Hamilton R, Cadenas E, Brinton RD. Ovarian hormone loss induces bioenergetic deficits and mitochondrial β -amyloid. *Neurobiol Aging*. 2012;33(8):1507–1521.