

Peer-Review

Majumdar, Nainika. 2026. "Testing the Critical Window Hypothesis in Alzheimer's Disease: Brain vs Gut Mechanisms of Estrogen Therapy Timing." *Journal of High School Science* 10 (1): 292–314. <https://doi.org/10.64336/001c.158816>.

The paper presents content already in the public domain - claims of the intersection of the gut microbiome, estrogen and AD notwithstanding. Therefore, the work does not contribute significantly to the corpus of existing knowledge in the field. It also does not satisfy the Journal's expectations for a review paper as seen here: <https://jhss.scholasticahq.com/for-authors> , types of manuscripts, review papers.

If you are interested in the intersection, then you will need to answer (or at least address) the following questions in the manuscript: why does estrogen stop acting to decrease AD if administered post 5 years after menopause? Is it because, at that time, it cannot alter the gut microbiota ? (why?). Is it because, the gut-brain axis has been inundated with 'leaky' inflammatory signals such that no pharmacological amount of estrogen can reverse it? Is it because gut dysbiosis is not as important a factor in the post-menopausal development of AD as thought? Is it because the gut microbiota control the enterohepatic estrogen circulation and recycling and dysbiosis interrupts or distorts this process? If this is the case, then a post-menopausal woman with gut dysbiosis should not respond to estrogen with respect to AD; regardless of whether estrogen is administered within 5 years of menopause. Please thoroughly research the literature and provide insights into these (and other intersection related) questions. Addressing these questions will increase the depth of the manuscript and contribute to the field. I can review it again after these questions have been satisfactorily addressed.

The reviewer raised several mechanistic questions regarding the loss of Alzheimer's disease (AD) risk reduction when estrogen-based hormone therapy (HT) is initiated more than five years after menopause. Below, I detail where and how each concern was addressed in the revised manuscript. Slight changes were also made to the Abstract and Discussion sections to reflect these revisions and ensure consistency within the entire paper.

1. Why does estrogen stop acting to decrease Alzheimer's disease risk if hormone therapy is administered more than five years after menopause?

Location of revision: Section 7.1 (Brain Mechanisms)

Revision made: I added a detailed brain-centered mechanistic explanation describing how prolonged estrogen deprivation leads to neuronal downregulation of estrogen receptors, including ER α , ER β , and GPER1. The revision explains that this receptor loss reduces both the number and functional responsiveness of estrogen receptors, limiting hormone therapy's ability to activate genomic and non-genomic neuroprotective signaling pathways. I further describe how this impaired signaling reduces synaptic maintenance, anti-inflammatory responses, mitochondrial

regulation, and cholinergic support. Additionally, I explain that prolonged estrogen deprivation is associated with a shift away from glucose-based metabolism toward ketone reliance, reflecting reduced neuronal metabolic flexibility and further limiting estrogen responsiveness.

Rationale: These receptor and metabolic changes provide a biologically supported explanation for the critical window hypothesis, demonstrating why estrogen therapy loses neuroprotective efficacy when initiated after prolonged estrogen deprivation.

2. Is this loss of benefit because, after that time, estrogen can no longer alter the gut microbiota? If so, why?

Location of revision: Section 7.2 (Gut Mechanisms)

Revision made: I addressed this question by evaluating whether estrogen loses its ability to modulate the gut microbiome after prolonged deprivation. Rather than assuming time-dependent gut irreversibility, I cite empirical evidence. Specifically, I reference data from the REIMAGINE study showing that estrogen-based hormone therapy reduces gut dysbiosis and increases microbial diversity in postmenopausal women. Although the study does not stratify participants by years since menopause, it does not indicate diminished gut responsiveness to estrogen at later postmenopausal stages. I further support this conclusion by citing Kaliannan et al. (2018), which demonstrated that 17 β -estradiol alters gut microbial composition in ovariectomized mice, a model of long-term estrogen absence.

Rationale: These findings indicate that estrogen retains gut-modulatory effects even after prolonged deprivation, suggesting that loss of gut responsiveness is unlikely to explain the critical window effect observed for Alzheimer's disease risk reduction.

3. Is it because the gut–brain axis has already been inundated with chronic “leaky gut” inflammatory signals, such that no pharmacological amount of estrogen can reverse it?

Location of revision: Section 8.1 (Inflammation)

Revision made: I addressed this hypothesis by acknowledging its biological plausibility while clearly delineating its evidentiary limitations. The revision explains that prolonged estrogen deficiency may compromise gut barrier integrity, increasing intestinal permeability and systemic inflammatory signaling that could contribute to neurodegeneration. However, I explicitly state that there is currently no direct human evidence demonstrating that late-stage gut–brain axis inflammation is irreversible or unresponsive to estrogen therapy.

Rationale: By distinguishing between plausible mechanisms and data from prior studies, I clarify that chronic inflammation may modify hormone therapy outcomes but is unlikely to be the primary mechanism underlying the loss of therapeutic benefit beyond the critical window.

4. Is it because the gut microbiota regulate enterohepatic estrogen circulation and recycling (the estrobolome), and dysbiosis interrupts or distorts this process?

Location of revision: Section 8.2 (Enterohepatic Circulation)

Revision made: I examined the role of the gut microbiota in estrogen metabolism and recycling by discussing how sustained dysbiosis may impair the estrobolome's ability to deconjugate and recycle estrogen. However, I argue that even if enterohepatic circulation is disrupted, pharmacological estrogen administration can bypass microbial recycling because hormone therapy delivers estrogen independently of endogenous microbial metabolism.

Rationale: This reasoning suggests that while estrobolome disruption may influence estrogen bioavailability, it does not sufficiently explain the complete loss of estrogen responsiveness observed with late hormone therapy initiation.

5. If this estrobolome disruption is the case, then would a postmenopausal woman with gut dysbiosis fail to respond to estrogen therapy with respect to AD prevention—regardless of whether estrogen is administered within five years of menopause?

Location of revision: Section 8.2 (Enterohepatic Circulation)

Revision made: I addressed this implication directly by reasoning that if gut dysbiosis alone were sufficient to abolish estrogen responsiveness, then hormone therapy would fail even when administered within the critical window. However, studies show that estrogen therapy remains effective in reducing Alzheimer's disease risk when initiated early post-menopause, even in populations where gut dysbiosis is present.

Rationale: This indicates that estrobolome disruption cannot independently determine hormone therapy failure and reinforces the importance of timing-dependent neuronal estrogen receptor integrity.

6. Is it because gut dysbiosis is not as important a factor in postmenopausal Alzheimer's disease development as previously believed?

Location of revision: Sections 7.2 and 8

Revision made: Rather than dismissing the gut microbiome's role in postmenopausal Alzheimer's disease, I reframed gut dysbiosis as contributory rather than causative with respect to hormone therapy efficacy. I show that estrogen can continue to modulate the gut microbiome after prolonged deprivation and that improvements in gut dysbiosis do not restore Alzheimer's disease protection when hormone therapy is initiated late.

Rationale: This supports the conclusion that while gut dysbiosis remains an important factor in postmenopausal Alzheimer's disease risk, it functions as a secondary modifying factor for hormone therapy outcomes, whereas brain-centered mechanisms—particularly estrogen receptor signaling and metabolic flexibility—are the primary drivers of the critical window effect.

Thank you for addressing my comments so comprehensively. Much appreciated. The manuscript is much improved from the earlier iteration. As a last step, please describe the following three literature content deficiencies in the manuscript.

1. Add a short paragraph that explicitly identifies quantitative gaps in the literature (missing quantitative relationships in the literature), e.g.:

There are no studies on Time-course of ER α /ER β decline in human hippocampus

There are no studies/data on Threshold levels of receptor density below which HT efficacy collapses

There are no studies that Quantify the interaction between estrogen dose, timing, and neuroinflammatory load in the literature.

2. Add a paragraph that describes and summarizes methodological deficiencies in the literature.

“Current limitations in the literature stem from:

(i) cross-sectional designs,

(ii) lack of timing stratification,

(iii) absence of multimodal gut–brain measurements, and

(iv) overreliance on animal models for mechanistic inference.”

3. Propose an actual experimental design which treats the “critical window” not as a vague epidemiologic timing effect, but as a state-dependent biological responsiveness problem and directly tests whether the gut and brain remain estrogen-responsive on different timelines. This experimental design should challenge the implicit assumption that all estrogen targets lose responsiveness together, extract new mechanistic insight from existing HT, microbiome, and neuroimaging literature, produce falsifiable predictions and is implementable with existing clinical research infrastructure.

The proposed actual experimental design is described below (courtesy of chatgpt, which I agree with). You would only need to modify the language to write in your own word and insert into the manuscript.

“A directly testable approach to disentangle gut- versus brain-centered mechanisms of the critical window hypothesis would be a timing-stratified estrogen intervention trial in postmenopausal women. Participants could be stratified by years since menopause (e.g., early: <3 years; mid: 5–8 years; late: ≥ 10 years) and randomized to estrogen therapy or placebo. Longitudinal assessments would integrate gut microbiome composition and estrobolome activity, circulating estrogen metabolites, markers of gut permeability and systemic inflammation, and neurobiological endpoints including cerebral glucose metabolism, neuroinflammatory biomarkers, and cognitive performance. If gut microbiome responsiveness to estrogen is preserved across all timing strata while

neurobiological responsiveness is selectively blunted in late initiators, this would provide direct experimental support for brain-centered receptor and metabolic mechanisms underlying the critical window phenomenon.”

I look forward to your last iteration.

The reviewer identified three remaining literature deficiencies related to (i) missing quantitative relationships, (ii) methodological limitations in existing studies, and (iii) the need for a directly testable experimental design addressing the critical window hypothesis. Below, I specify where and how each concern was addressed in the revised manuscript. Minor wording adjustments were also made throughout the Concluding Remarks to ensure consistency with these additions.

7. Add a short paragraph that explicitly identifies quantitative gaps in the literature (missing quantitative relationships in the literature), e.g.:

- **There are no studies on time-course of ER α /ER β decline in human hippocampus**
- **There are no studies/data on Threshold levels of receptor density below which HT efficacy collapses**
- **There are no studies that quantify the interaction between estrogen dose, timing, and neuroinflammatory load in the literature.**

Location of revision: Section 10, paragraph 2 (“Beyond these conceptual gaps, there are also quantitative gaps in this field.”)

Revision made: I added a dedicated paragraph explicitly describing missing quantitative relationships in the current literature. This section notes the absence of longitudinal data characterizing the time-course of estrogen receptor (ER α /ER β) decline in the human hippocampus during menopause, the lack of defined receptor-density thresholds below which hormone therapy efficacy diminishes, and the absence of studies quantifying interactions among hormone therapy dosage, timing of initiation, neuroinflammatory burden, and gut microbial diversity.

Rationale: Identifying these missing measurable relationships clarifies that current limitations are not only conceptual but also quantitative, and highlights the need for studies capable of producing predictive, clinically testable parameters.

8. Add a paragraph that describes and summarizes methodological deficiencies in the literature. “Current limitations in the literature stem from:

- (i) cross-sectional designs,**
- (ii) lack of timing stratification,**
- (iii) absence of multimodal gut–brain measurements, and**

(iv) overreliance on animal models for mechanistic inference.”

Location of revision: Section 10, paragraph 3 (“In terms of methodology...”)

Revision made: I added a focused discussion of methodological weaknesses across the existing literature. This section emphasizes the predominance of cross-sectional or retrospective designs, the scarcity of long-term longitudinal trials, limited timing stratification by years since menopause, the lack of multimodal gut–brain measurements within the same participants, and the heavy reliance on animal models for mechanistic inference.

Rationale: Summarizing these recurring design limitations clarifies why causal conclusions remain uncertain and explains the difficulty in directly testing timing-dependent estrogen responsiveness in humans.

- 9. Propose an actual experimental design which treats the “critical window” not as a vague epidemiologic timing effect, but as a state-dependent biological responsiveness problem and directly tests whether the gut and brain remain estrogen-responsive on different timelines. This experimental design should challenge the implicit assumption that all estrogen targets lose responsiveness together, extract new mechanistic insight from existing HT, microbiome, and neuroimaging literature, produce falsifiable predictions and is implementable with existing clinical research infrastructure. The proposed actual experimental design is described below (courtesy of chatgpt, which I agree with). You would only need to modify the language to write in your own word and insert into the manuscript.**

“A directly testable approach to disentangle gut- versus brain-centered mechanisms of the critical window hypothesis would be a timing-stratified estrogen intervention trial in postmenopausal women. Participants could be stratified by years since menopause (e.g., early: <3 years; mid: 5–8 years; late: ≥10 years) and randomized to estrogen therapy or placebo. Longitudinal assessments would integrate gut microbiome composition and estrobolome activity, circulating estrogen metabolites, markers of gut permeability and systemic inflammation, and neurobiological endpoints including cerebral glucose metabolism, neuroinflammatory biomarkers, and cognitive performance. If gut microbiome responsiveness to estrogen is preserved across all timing strata while neurobiological responsiveness is selectively blunted in late initiators, this would provide direct experimental support for brain-centered receptor and metabolic mechanisms underlying the critical window phenomenon.”

Location of revision: Section 10.1 (“Proposed Experimental Test of the Critical Window Hypothesis”)

Revision made: I introduced a new subsection proposing a timing-stratified, randomized estrogen intervention trial in postmenopausal women. Participants are grouped by years since menopause (early, mid, late) and randomized to estrogen therapy or placebo. Longitudinal outcomes integrate gut microbiome composition and estrobolome activity, circulating estrogen metabolites, markers of gut permeability and inflammation, and neurobiological measures including FDG-PET brain metabolism, neuroinflammatory biomarkers, MRI measures, and cognitive testing. The design includes explicit, falsifiable predictions to determine whether gut and brain tissues lose estrogen responsiveness simultaneously or on distinct timelines.

Rationale: This design reframes the critical window as a state-dependent biological responsiveness problem rather than a purely epidemiologic observation, and provides a practical, implementable framework capable of generating mechanistic insight.

Thank you for addressing my comments. As a last revision, minor inconsistencies of over-reach remain. Please include language/limitations/tone down over-reach.

1.If you have not already, [1] please explicitly state in your limitations section that microbiome evidence is exploratory, not conformational, due to small sample (REIMAGINE, n=35) used as strong support and animal models used to infer human timing effects. [2] heterogeneity regarding age stratification beyond timing, genetic risk (e.g., APOE ε4), lifestyle factors and baseline microbiome variability are confounding variables which have not been adequately discussed in this report. [3] The statistical plan did not include interaction testing, longitudinal modeling and multiple hypothesis correction [4] The The asymmetry in confidence (brain mechanism is strongly supported while gut mechanism is unlikely primary driver) is not justified by evidence quality because gut evidence is based on small (35#) human study + animal evidence and brain mechanism is largely mechanistic and indirect. [5] causal over-reach such as "“estrogen-only HT... has been found to reduce Alzheimer’s risk” needs to be toned down

“[1] please explicitly state in your limitations section that microbiome evidence is exploratory, not conformational, due to small sample (REIMAGINE, n=35) used as strong support and animal models used to infer human timing effects.”

Location of revision: Section 10 (Limitations), paragraph discussing microbiome evidence and the REIMAGINE study (n=35).

Revision made: I explicitly stated that the microbiome evidence presented in this review is exploratory rather than confirmatory. I clarified that the REIMAGINE human cohort (n=35) is small and therefore insufficient to draw definitive conclusions. I also acknowledged that several timing-related inferences are derived from animal models and may not directly translate to human physiology.

Rationale: This revision ensures that microbiome findings are accurately characterized as preliminary and hypothesis-generating rather than definitive, directly addressing the concern of over-interpreting limited human data.

10. “[2] heterogeneity regarding age stratification beyond timing, genetic risk (e.g., APOE ε4), lifestyle factors and baseline microbiome variability are confounding variables which have not been adequately discussed in this report.”

Location of revision: Section 10 (Limitations), paragraph addressing heterogeneity and confounders.

Revision made: I added a detailed discussion acknowledging heterogeneity beyond simple early-versus-late timing. Specifically, I addressed age stratification differences, APOE ε4 genetic risk status, lifestyle variables (such as diet, exercise, and metabolic health), and baseline microbiome variability as potential confounding factors that may influence study outcomes.

Rationale: By explicitly recognizing these variables, I clarified that observed associations cannot be attributed solely to hormone timing and that individual biological and environmental variability limits interpretability across studies.

11. “[3] The statistical plan did not include interaction testing, longitudinal modeling and multiple hypothesis correction.”

Location of revision: Section 10 (Limitations), statistical limitations paragraph.

Revision made: I explicitly noted that many of the cited studies did not include interaction testing (ex: timing × genotype effects), longitudinal modeling to record changes over time, or appropriate multiple hypothesis testing adjustments.

Rationale: This addition strengthens methodological transparency and acknowledges that the absence of these statistical approaches limits causal inference and interpretive strength.

12. “[4] The asymmetry in confidence (brain mechanism is strongly supported while gut mechanism is unlikely primary driver) is not justified by evidence quality because gut evidence is based on small (35#) human study + animal evidence and brain mechanism is largely mechanistic and indirect.”

Location of revision: Discussion section comparing brain-centered and gut-centered mechanisms; reinforced in Limitations

Revision made: I recalibrated the tone of the comparative analysis between brain and gut mechanisms. I removed language that strongly dismissed gut mechanisms as unlikely primary drivers and instead acknowledged that both brain-centered and gut-centered explanations rely largely on mechanistic, indirect, or small-sample evidence. I presented both pathways as plausible but still under investigation.

Rationale: This restores balance in evidentiary weighting and ensures that confidence levels more accurately reflect the quality and type of available evidence.

13. “[5] causal over-reach such as ‘estrogen-only HT... has been found to reduce Alzheimer’s risk’ needs to be toned down.”

Location of revision: Results and Discussion sections where hormone therapy outcomes are described.

Revision made: I revised causal phrasing throughout the manuscript, replacing statements such as “has been found to reduce Alzheimer’s risk” with more accurate associative language (ex: “is associated with reduced risk,” “has been linked to,” or “may reduce risk depending on timing”).

Rationale: This ensures that conclusions align with the strength of the evidence and avoids implying causation where studies primarily demonstrate association.

14. “Please include language/limitations/tone down over-reach.”

Location of revision: Throughout the manuscript (Abstract, Results, Discussion, and Limitations sections).

Revision made: I corrected spelling and typographical errors throughout the manuscript and refined language to reduce overreach. I adjusted wording that implied certainty or definitive mechanistic conclusions and replaced it with more cautious, evidence-aligned phrasing. I also improved clarity and consistency in tone across sections.

Rationale: These revisions ensure the manuscript maintains an academically appropriate tone, accurately reflects evidentiary strength, and improves overall clarity.

Thank you for addressing my comments. Accept.

However, I still need you to reformat the references section per APA format. Your DOI links need to be live as well. Please submit a word.doc or libreoffice writer file properly formatted so we may begin copyediting.