

RESPONSE TO REVIEWERS

Manuscript ID: neurosci-4559452

Manuscript: “Early Time-Restricted Eating, Circadian Alignment, and Cognitive Preservation in Older Adults: A Neurobiological Hypothesis”

Authors: Bianca Farias Lima, Gioconda Ferreira, Fernanda de Oliveira Calvente, Yasmin da Silva Costa, Mariana Alencar, Jhenifer de Paiva Quadros and Hércules Rezende Freitas

General note to the Editor and Reviewers. We thank the Editor and the three Reviewers for their careful reading and constructive comments. We have revised the manuscript extensively. The main changes are: (i) a new Section 2 describing how the hypothesis was developed, the exact input given to the AI co-scientist (reproduced verbatim in the new Supplementary File S1), what the system contributed, and how the authors verified it; (ii) a new Table 1 that grades every link of the causal chain as established, biologically plausible, or hypothesis-specific; (iii) an expanded and more nuanced account of glymphatic biology, including ageing, vascular factors, and contrary evidence; (iv) the Dual-Gating model is now explicitly labelled as hypothesized, with primary references for each step; (v) sleep is now modelled as a potential mediator and effect modifier (revised Figure 1); (vi) a new Section 3.4 on nutrient-sensing (insulin/IGF-1, mTOR, AMPK, autophagy) and gut microbiota pathways; (vii) a substantially expanded trial section with dietary controls, safety monitoring for sarcopenia, an assessment schedule (new Table 3), a justification of the 12-month duration, an explicit sample-size calculation, and a separate simulation-based power analysis for mediation; (viii) a harmonized definition of eTRE (16:00 cut-off); and (ix) more cautious language throughout, including removal of the “fifteenth risk factor” claim.

During revision we re-checked every reference against its PubMed record or, for the two items not indexed in PubMed, the publisher’s record. This identified and corrected several

errors in the original submission. (i) The review previously cited as “Cienfuegos et al., 2020” is by Adafer et al. (ii) The author lists of Dashti et al. (2025), van der Zwet et al. (2025), García Cobarro et al. (2025), Hablitz et al. (2020), Zhao et al. (2025), Franzago et al. (2023), and Currenti et al. (2021) were corrected, and the article number of Dashti et al. is 385. (iii) The reference previously listed as “Froy, 2011” did not match its cited source and was replaced by the primary study it was meant to support (Damiola et al., 2000). (iv) Most importantly, the original manuscript misreported Currenti et al. (2021) as a null study. In fact, that cross-sectional study found that an eating window shorter than 10 hours, and eating breakfast, were associated with lower odds of cognitive impairment. The text (Sections 4 and 4.1) and Table 2 now report this finding correctly, together with its limitations and with the one contrary study identified by the scoping review of Ding et al. (2025). (v) We removed study details that we could not confirm from the published record (the sample size and randomization details given for Zhao et al., 2025) and now describe the Dashti et al. cohort as in the article (repeated assessments between 1983 and 2017). The reference list now contains 72 entries, numbered in order of first citation.

Section numbers, tables, and figures in our responses refer to the clean revised manuscript. All changes are shown in the accompanying version with tracked changes (added text in blue, deleted text in red). Because the reference list was reordered, reference numbers in the tracked-changes file differ from those in the clean file.

REVIEWER 1

This is a thought-provoking manuscript addressing an emerging intersection between chrononutrition, circadian biology, brain clearance, and cognitive aging. The effort to formulate a falsifiable biological hypothesis and translate it into a proposed experimental design is a strength. However, substantial revision is required to improve mechanistic rigor, distinguish established evidence from speculation, and strengthen the treatment of glymphatic biology.

Response: We thank the Reviewer for this positive and constructive assessment. We agree that the original version did not separate established evidence from speculation clearly enough. We have addressed each point below; the main changes are summarized in the general note above.

Here are my comments/ suggestions:

1. The central causal argument should be presented more cautiously.

The manuscript proposes that eTRE protects cognition through circadian synchronization of glymphatic clearance, neuroimmune rhythmicity, and synaptic plasticity. However, much of the supporting evidence is indirect, preclinical, or derived from adjacent fields rather than studies directly testing eTRE → glymphatic function → cognition in older adults. The authors appropriately acknowledge that the glymphatic mechanism has not yet been directly validated in randomized trials, but this qualification should be more consistently reflected throughout the manuscript.

I suggest distinguishing clearly between established evidence, biological plausibility, and hypothesis-specific predictions.

Response 1.1: We agree. We now use an explicit three-level evidence convention, defined at the end of the Introduction: established evidence, biological plausibility, and hypothesis-specific predictions. A new Table 1 (“Evidence status of each link in the proposed causal chain”) applies this convention to each link, with the main supporting references and the main remaining gap. The text now states repeatedly that no link has been established for meal timing in humans (for example, the Figure 1 legend and Sections 3.2, 4, and 4.1). The hypothesis statement itself was softened (Section 3): eTRE is proposed to act through mechanisms “at least partly separable” from caloric intake, diet composition, and weight loss, and we no longer claim independence from sleep. The Abstract and Conclusions were revised in the same way.

2. The glymphatic discussion requires greater mechanistic depth and nuance.

The manuscript currently describes the glymphatic system rather simply as an “AQP4-dependent perivascular network” responsible for clearance of A β and phosphorylated tau. This section would benefit from discussing the broader neurovascular/perivascular context, including astrocytic endfeet and AQP4 polarization, perivascular-space integrity, vascular pulsatility, sleep-dependent CSF–ISF dynamics, and age-related alterations in these processes.

This is particularly important because the target population is ≥ 65 years, in whom vascular remodeling, cerebral small-vessel pathology, enlarged perivascular spaces, and altered AQP4 organization may independently influence clearance efficiency.

Response 1.2: We thank the Reviewer for this important suggestion. The description of the glymphatic pathway (Section 3.2) was rewritten and expanded. It now covers: CSF–ISF exchange along periarterial and perivenous routes; AQP4 polarization at astrocytic endfeet (Iliff et al., 2012); sleep-dependent expansion of the interstitial space (Xie et al., 2013); arterial pulsatility as a driver of perivascular flow (Mestre et al., 2018); NE-driven slow vasomotion during NREM sleep (Hauglund et al., 2025); and coupled neural, haemodynamic, and CSF oscillations in human sleep (Fultz et al., 2019). A new paragraph addresses ageing: loss of AQP4 polarization and reduced perivascular exchange in aged mice (Kress et al., 2014); the association between perivascular AQP4 localization, AD pathology, and cognition in human brains (Zeppenfeld et al., 2017); arterial stiffening and hypertension; and small-vessel disease and enlarged perivascular spaces (Wardlaw et al., 2020). We explain that these factors could limit or modify any benefit of meal timing in people aged ≥ 65 years. For this reason, vascular burden now appears as an effect modifier in Figure 1, and perivascular-space burden and white-matter hyperintensities are measured and adjusted for in the trial (Sections 5.4 and 5.5). We also acknowledge that the model is debated (Miao et al., 2024) and that most direct evidence comes from rodents.

3. The proposed “Dual-Gating” model is interesting but currently appears too definitive.

The autonomic and metabolic gates are presented as specific biological mechanisms explaining the superiority of eTRE over ITRE. In particular, statements linking pre-sleep fasting to norepinephrine withdrawal and reduced perivascular hydraulic resistance, and nocturnal insulin reduction to greater IDE availability for central A β clearance, require stronger direct evidence.

I recommend explicitly labeling these pathways as hypothesized mechanistic links rather than established consequences of eTRE and providing direct references for each mechanistic step.

Response 1.3: We agree. Section 3.3 is now titled “The Dual-Gating Model (Hypothesized)”, and each gate is labelled as hypothesized in the text, in Figure 1, and in Table 4. Each step now has direct references, and each gate ends with a statement of the missing evidence. For the autonomic gate: food intake raises sympathetic nerve activity in humans (Fagius and Berne, 1994); NE regulates interstitial space volume (Xie et al., 2013); and NE oscillations drive glymphatic flow during NREM sleep (Hauglund et al., 2025). We state that no study has shown that meal timing changes brain NE dynamics or perivascular flow in humans. For the metabolic gate: insulin and A β compete for IDE (Qiu et al., 1998); IDE deficiency raises brain A β (Farris et al., 2003); raising insulin levels increases CSF A β 42 in older adults (Watson et al., 2003); and late meals produce larger glycaemic and insulin responses (Poggiogalle et al., 2018; Vujović et al., 2022). We state that this mechanism has not been shown in the human brain at physiological insulin levels. We also removed the phrase “reduced perivascular hydraulic resistance” because it went beyond the evidence. Table 1 lists both gates as “Hypothesized”.

4. Sleep should not be treated simply as an independent confounder.

The DAG treats sleep quality as a confounder rather than a mediator. Yet eTRE could itself modify sleep timing, sleep architecture, autonomic activity, melatonin/cortisol rhythms, or slow-wave sleep, which could subsequently influence glymphatic function and cognition.

The authors should therefore justify this causal assumption more carefully. A more realistic model may consider sleep as a potential mediator, confounder, or effect modifier, depending on the specific pathway being tested.

Response 1.4: We fully agree, and we thank the Reviewer for this point. We have revised the causal model. The new paragraph “Sleep: confounder, mediator, or effect modifier” (Section 4.1) explains that the role of sleep depends on when it is measured. Sleep before randomization is a confounder in observational studies and a baseline covariate in a trial. Sleep during the intervention may be changed by eTRE (for example, through sleep timing, slow-wave sleep, autonomic tone, or melatonin and cortisol rhythms; Crispin et al., 2011) and is therefore a potential mediator, especially for the autonomic gate. Sleep may also modify the effect, because clearance benefits may require adequate slow-wave sleep. We note that adjusting for on-intervention sleep as a confounder would remove part of the effect of interest. Figure 1 now shows on-intervention sleep on a separate dotted path as a potential mediator or modifier, and only baseline sleep among the confounders. In the analysis plan (Section 5.7), on-intervention sleep is included as a parallel mediator, baseline sleep as a covariate, and sleep is tested as an effect modifier in pre-specified interaction analyses.

5. DTI-ALPS should not be presented as a direct measurement of glymphatic flux.

The authors appropriately call DTI-ALPS an “indirect estimate,” but elsewhere the proposed interpretation approaches direct measurement of glymphatic function. DTI-ALPS is an indirect MRI-derived surrogate and may be affected by white-matter microstructure and vascular pathology.

The manuscript should consistently use terminology such as “imaging surrogate associated with glymphatic/perivascular function” and acknowledge its methodological limitations.

Response 1.5: We agree. We now describe DTI-ALPS consistently as “an MRI surrogate that is associated with perivascular and glymphatic function” that “does not measure

glymphatic flux”. The index is now a mechanistic outcome, not a primary outcome. A new Section 5.5 (“Interpreting DTI-ALPS”) explains how the index is derived (Taoka et al., 2017) and its limitations (Ringstad, 2024; Taoka et al., 2024). These include the small deep white-matter region measured, the small fraction of tissue occupied by perivascular spaces, and sensitivity to white-matter microstructure, fibre geometry, vascular pathology, age, and acquisition and processing choices. To address these limitations, DTI-ALPS will be analysed together with free-water and white-matter integrity metrics, with perivascular-space burden and white-matter hyperintensities as covariates and harmonized protocols. Conclusions about clearance will require agreement with fluid biomarkers (p-tau217, NfL).

6. The proposed RCT is conceptually strong but requires refinement.

The eTRE versus ITRE versus habitual-eating design is a major strength because it attempts to separate fasting duration from circadian timing. However, the authors should clarify how total caloric intake, diet quality, macronutrient composition, weight loss, metabolic improvement, medication changes, chronotype, and sleep duration will be controlled. Otherwise, any cognitive or biomarker effect cannot confidently be attributed to meal timing itself.

Response 1.6: We thank the Reviewer. A new Section 5.3 (“Control of Diet, Weight, and Co-Interventions”) now explains how each factor will be handled. All arms receive weight-maintenance energy prescriptions, the same dietary-quality guidance, and meal templates with matched macronutrient composition. Body weight is checked every 2 weeks, and energy advice is adjusted to keep weight within $\pm 2\%$ of baseline. Residual changes in weight, HbA1c, and HOMA-IR are modelled as competing mediators. Diet quality is scored from repeated 24-hour recalls, and medication changes are recorded at every visit. Physical activity and sleep duration are monitored by actigraphy, and biological samples are collected at a fixed clock time after a standardized fast. Randomization is now stratified by chronotype (MEQ) and cognitive status (Section 5.1), and adherence is defined operationally and verified with time-stamped photo logs and, in a subsample, continuous glucose monitoring. Protein intake, frailty, and lean mass are addressed in Section 5.2 (see Response 1.8).

7. The sample-size justification needs substantially more detail.

The manuscript proposes approximately 80–120 participants per arm based on $d \approx 0.3$, 80% power, and anticipated attrition. Given the three-arm longitudinal design, repeated biomarker measurements, multiple cognitive outcomes, and especially the proposed SEM/mediation analysis, a conventional pairwise effect-size calculation may be insufficient.

The authors should specify the primary endpoint on which the power calculation is based and provide a separate justification for the mediation/SEM analysis.

Response 1.7: We agree, and we thank the Reviewer for prompting a more rigorous calculation. On re-examination, the original range (80–120 per arm with $d \approx 0.3$) was not explained well enough: a simple two-sample t-test with $d = 0.3$ would require about 175 participants per arm. Section 5.7 now specifies the primary endpoint for the sample size: the 12-month change in PACC-5 in the eTRE-versus-ITRE comparison, analysed by ANCOVA adjusted for baseline. With $d = 0.3$, two-sided $\alpha = 0.05$, 80% power, and a baseline–12-month correlation of $\rho = 0.7$ (to be confirmed in the pilot), the formula $n = 2(z_{1-\alpha/2} + z_{1-\beta})^2(1 - \rho^2)/d^2$ gives about 90 evaluable participants per arm. This corresponds to 120 randomized per arm ($N = 360$) with 25% attrition, or about 130 per arm with 30% attrition. The eTRE-versus-habitual comparison is tested only after the primary comparison is significant (fixed-sequence testing), so no further α adjustment is needed.

We now also give a separate justification for the mediation analysis. Using a Monte Carlo simulation (2000 replicates, joint-significance test, 90 evaluable participants per arm), we found $\geq 80\%$ power when the intervention-to-mediator path is ≥ 0.45 SD and the mediator-to-outcome path is ≥ 0.25 . Power was 76% for paths of 0.40 and 0.30, and about 50% for an intervention-to-mediator path of 0.30. If the pilot suggests a path of about 0.35, about 140–150 evaluable participants per arm would be required. The final sample size will therefore be the larger of the two estimates, recalculated by simulation from the pilot

parameters (Fritz and MacKinnon, 2007; Schoemann et al., 2017). We also state that the mediation analysis is secondary and that, because mediators are not randomized, its results will be interpreted as supportive rather than conclusive.

8. The sarcopenia issue deserves greater attention.

The manuscript appropriately recognizes that compressed feeding windows could create nutritional risk in older adults and recommends protein intake of 1.2–1.6 g/kg/day. I suggest expanding this discussion to include lean-mass monitoring, resistance activity, distribution of protein across meals, frailty status, and exclusion/monitoring criteria for vulnerable participants.

Response 1.8: We agree, and we have expanded this topic in two places. Section 4.1 now cites the evidence on protein needs in older adults (Bauer et al., 2013; Deutz et al., 2014), on protein distribution across meals (Mamerow et al., 2014), and on lean-mass loss in a TRE trial (Lowe et al., 2020). We also corrected the protein targets to match these guidelines. A new Section 5.2 (“Participants and Safety Monitoring”) adds: exclusion of frail participants (Fried phenotype) and of those with probable or confirmed sarcopenia (EWGSOP2), low BMI, recent weight loss, or hypoglycaemia-prone therapy; individualized protein targets (1.0–1.2 g/kg/day, or 1.2–1.5 g/kg/day for those with chronic disease or low muscle mass); about 25–30 g of protein per main meal within the window; the same resistance and aerobic activity guidance in all arms; DXA lean mass at 0, 6, and 12 months; handgrip strength and SPPB every 3 months; and pre-specified stopping rules (weight loss >5%, handgrip or SPPB below sarcopenia cut-offs, or recurrent hypoglycaemia).

9. The claim of a potential “fifteenth modifiable risk factor” is premature.

The manuscript suggests that positive findings could advance meal timing toward recognition as a fifteenth modifiable dementia risk factor. This is an interesting conceptual proposition, but the current human evidence remains limited. I recommend moderating this language and emphasizing that meal timing should first be considered a candidate behavioral exposure requiring prospective validation.

Response 1.9: We agree. The claim was removed from the Abstract, Section 6, and the Conclusions. Section 6 now states that meal timing does not yet meet the Commission's standards (no consistent prospective data on cognitive outcomes and no estimate of attributable fraction). It describes meal timing as "a candidate behavioural exposure that requires prospective validation", and it says only that further evidence would help judge whether meal timing should "eventually be considered" as a modifiable risk factor. The Conclusions now describe meal timing as "a biologically plausible but untested candidate modifiable exposure".

10. The role of AI in generating the hypothesis needs careful framing.

The manuscript states that the Dual-Gating model, three-arm RCT, and SEM criterion emerged from an AI co-scientist analysis and describes the work as a proof-of-concept for human–AI scientific hypothesis development. This is unusual and potentially interesting, but the scientific validity of the manuscript must derive from the literature and biological reasoning rather than the AI system itself. The authors should make clearer which elements were AI-generated, how they were independently verified by the authors, and whether all references and mechanistic propositions suggested by the system were manually validated.

Response 1.10: We agree that the scientific case must rest on the literature and on biological reasoning. A new Section 2 ("Hypothesis Development and Use of Generative AI") now describes the process. Section 2.1 describes the human-authored thesis draft, which already contained the operational definition, the three pathways, the DAG, the main outcomes, and a two-arm trial. Section 2.2 describes the AI co-scientist run: the tool, date, and data sources, and the authors' exact prompt, which the system records as the Research Summary of the run specification. The complete run specification, including this prompt, is reproduced verbatim in Supplementary File S1. Section 2.2 also distinguishes what came from the prompt from what the system added at the goal-specification stage. The prompt contained the target population, the eTRE definition, the outcomes, and the requirement that any benefit be mediated by lymphatic or inflammatory change. The

three-arm comparison (eTRE versus ITRE versus habitual eating) was introduced by the system among the run preferences. Section 2.3 states what the system contributed: the three-arm design, the Dual-Gating model, the feasibility pilot, standardized sampling times, and the use of SEM for mediation. It also names system-generated mechanisms that we rejected because their steps could not be traced to primary evidence, and it describes our verification. Every mechanistic step was checked against independently identified primary literature, every reference was checked manually, and unsupported links are labelled as hypothesized (Table 1). The section ends by stating that the scientific case rests on the cited literature and on the authors' reasoning. The proof-of-concept paragraph in Section 6 was rewritten in more modest terms: it now describes the AI output as a source of candidate ideas, not of evidence, and notes that several proposals were rejected on review.

11. The authors should standardize the use of TRE versus TRF, clarify why 19:00 is used in the operational definition whereas the proposed experimental eTRE arm uses 08:00–16:00, and ensure that statements about “independence” from caloric intake or sleep are not stronger than the evidence allows. The manuscript would also benefit from a dedicated limitations paragraph summarizing translational gaps between animal lymphatic studies and human chrononutrition research.

Response 1.11: We thank the Reviewer. (a) Terminology: we now use TRE for human studies and TRF for animal studies, as stated at the end of the Introduction. We keep “TRF” only where the original human study used that term (Zhao et al., 2025, “termed TRF by the authors”). (b) 19:00 versus 16:00: the definition has been harmonized (Section 3.1; see also Response 3.1). eTRE is now defined as $\geq 80\%$ of daily energy before 16:00, a window of ≤ 10 hours, and ≥ 3 hours between the last intake and sleep. The trial prescribes 08:00–16:00, so the two definitions agree, and we explain why the earlier 19:00 cut-off was abandoned. (c) Independence: we removed claims of independence from caloric intake and sleep. The hypothesis now refers to effects that are “at least partly separable” from caloric intake, diet composition, and weight loss, and it explicitly allows sleep to carry part of the effect (Section 3). The description of Hatori et al. now says

“without reducing caloric intake”. (d) Limitations: Section 4.1 now includes a dedicated paragraph on translational gaps. It covers the rodent origin of most glymphatic evidence (nocturnal species with different sleep architecture and brain geometry), inconsistencies among rodent studies, the lack of direct non-invasive human measures, the absence of human studies linking meal timing to clearance, NE dynamics, or central IDE activity, the short duration and metabolic endpoints of most human TRE trials, and age-related vascular disease in the target population.

REVIEWER 2

Overall Evaluation: This manuscript presents a timely and potentially valuable hypothesis linking early time-restricted eating (eTRE) with cognitive preservation in older adults through circadian alignment, glymphatic clearance, neuroimmune rhythmicity, and hippocampal synaptic plasticity. The authors further propose a “Dual-Gating” model involving pre-sleep autonomic regulation and nocturnal insulin–IDE signaling and outline a three-arm randomized controlled trial to distinguish the effects of meal timing from fasting duration. The manuscript addresses an emerging intersection between chrononutrition, aging, and neurodegeneration. The proposed integration of glymphatic physiology and meal timing is particularly interesting, and the emphasis on falsifiable predictions is a strength. However, several issues currently limit the manuscript’s mechanistic rigor and translational reliability. Many of the proposed links remain inferential. The distinction between established findings, mechanistic extrapolation, and speculative hypotheses should be made more explicit. In particular, the causal relationship between eTRE and glymphatic function in humans remains unvalidated, and the proposed autonomic and IDE-mediated “gates” require stronger experimental justification. The treatment of sleep quality as a confounder rather than a potential mediator also requires reconsideration. In addition, the proposed RCT and mediation framework may be statistically overinterpreted, especially given the limitations of DTI-ALPS as an indirect glymphatic measure. The manuscript would benefit from a more balanced discussion of alternative pathways, including metabolic signaling, mTOR-related nutrient sensing, insulin signaling, autophagy, and gut–brain communication.

Overall, the manuscript contains a novel and stimulating conceptual framework, but clarification of evidence strength, mechanistic limitations, causal assumptions, and experimental feasibility is needed before publication.

Response: We thank the Reviewer for the careful evaluation and for recognizing the novelty of the framework. We agree with the general concerns. In the revised manuscript we (i) separate established findings, mechanistic extrapolation, and hypothesis-specific predictions in a new Table 1; (ii) state explicitly that the eTRE–glymphatic link is untested in humans; (iii) label the autonomic and IDE gates as hypothesized and give primary references for each step; (iv) model sleep as a potential mediator and effect modifier; (v) treat DTI-ALPS as an indirect surrogate and describe the mediation analysis as secondary, separately powered, and supportive rather than conclusive; and (vi) add a new Section 3.4 on nutrient sensing (mTOR, insulin/IGF-1, AMPK, autophagy) and on the gut–brain axis. Our point-by-point responses follow.

Major Comments:

The central premise relies heavily on the assumption that early meal timing enhances glymphatic clearance in humans, yet direct human evidence remains limited or absent. Please acknowledge this major knowledge gap and avoid presenting the glymphatic pathway as an already established consequence of eTRE.

Response 2.1: We agree. The revised manuscript states this gap in several places. Section 3.2 ends the glymphatic paragraph with “Whether meal timing changes glymphatic function in humans has not been tested”; Table 1 lists the “Benefit mediated by clearance/inflammation” link as a hypothesis-specific prediction with no supporting evidence; the Figure 1 legend states that none of the links has been established for meal timing in humans; and Section 4.1 lists this gap first among the translational gaps. The

Abstract, Section 3, and the Conclusions now describe the glymphatic route as a candidate pathway, not an established consequence of eTRE.

The proposed autonomic and metabolic gates are conceptually interesting but appear to be assembled primarily from indirect evidence. Please provide stronger literature support for the specific links between pre-sleep norepinephrine withdrawal, perivascular resistance, IDE activity, and central amyloid- β clearance.

Response 2.2: We thank the Reviewer. We agree that the gates rest on indirect evidence, and we now say so. Each step is now referenced to primary studies (Section 3.3 and Table 1). For the autonomic gate we cite postprandial sympathetic activation in humans (Fagius and Berne, 1994), NE-dependent regulation of interstitial space volume (Xie et al., 2013), and NE-driven vasomotion that propels CSF during NREM sleep (Hauglund et al., 2025). For the metabolic gate we cite competition between insulin and A β for IDE (Qiu et al., 1998), increased brain A β in IDE-deficient mice (Farris et al., 2003), increased CSF A β 42 after raising insulin levels in older adults (Watson et al., 2003), larger glycaemic and insulin responses to late meals (Poggiogalle et al., 2018; Vujović et al., 2022), and mTORC1-dependent control of insulin production by nutrients (Fan et al., 2022; Fan et al., 2026). Each gate now ends with a statement of the missing evidence, and both are labelled “Hypothesized”.

The proposed use of DTI-ALPS as a primary biological readout requires more cautious justification because it is an indirect and debated proxy for glymphatic activity. Please discuss its limitations, potential confounders, and whether additional imaging or fluid-based biomarkers would be necessary.

Response 2.3: We agree. DTI-ALPS is no longer a primary outcome; the primary biological outcome is now the plasma p-tau217 trajectory, and DTI-ALPS is a mechanistic outcome (Section 5.4). A new Section 5.5 discusses the method’s limitations and confounders (Ringstad, 2024; Taoka et al., 2024): the small deep white-matter region measured, and sensitivity to white-matter microstructure, fibre geometry, vascular pathology, age, and acquisition and processing choices. It also states that additional measures are needed: free-water and white-matter integrity metrics, perivascular-space

and white-matter hyperintensity burden as covariates, harmonized protocols, and agreement with fluid biomarkers (p-tau217, NfL). Where feasible, dynamic CSF-flow MRI during sleep could be used in a subsample. The assessment schedule is given in the new Table 3.

The mechanistic discussion would be strengthened by incorporating nutrient-sensing pathways, particularly mTOR signaling, insulin/IGF-1 signaling, AMPK, and autophagy, which are central regulators of aging, metabolism, proteostasis, and neurodegeneration. Please consider discussing relevant evidence, including PMID: 41528843, 35623230, and 41695773, to place the proposed chrononutrition model within the broader molecular framework of aging biology.

Response 2.4: We thank the Reviewer for this valuable suggestion and for the references. A new Section 3.4 (“Alternative and Parallel Pathways”) places the model within the molecular biology of ageing. It covers insulin/IGF-1 signalling, mTOR, and AMPK (Kenyon, 2010; Saxton and Sabatini, 2017; Herzig and Shaw, 2018) and their roles in autophagy, proteostasis, and mitochondrial function in dietary restriction and ageing. We cite the suggested review by Fan and Xu (2026; PMID 41695773) and López-Otín et al. (2023). We add evidence that the lifespan benefit of intermittent TRF requires circadian autophagy (Ulgherait et al., 2021) and that early TRE changes clock and autophagy gene expression in humans (Jamshed et al., 2019). The two other suggested studies (Fan et al., 2022, PMID 35623230; Fan et al., 2026, PMID 41528843) show that amino acids and nutrients control beta-cell insulin output through mTORC1. We cite them in the metabolic gate (Section 3.3) as a mechanistic link between the timing of nutrient load and nocturnal insulin exposure. These pathways also appear in Figure 1 as parallel or competing routes, and fasting insulin, glucose, HbA1c, and HOMA-IR are measured and modelled as competing mediators in the trial (Sections 5.3, 5.4, and 5.7).

The manuscript mentions the gut microbiota–propionic acid–FFAR3 pathway but does not integrate it into the proposed model. Given the emerging evidence that time-restricted feeding may influence cognition through microbiota-derived metabolites, this pathway should be discussed as a plausible parallel or competing mechanism.

Response 2.5: We agree. Section 3.4 now presents the gut pathway as a plausible parallel or competing mechanism. It notes that feeding schedules reshape daily rhythms of the gut microbiome (Zarrinpar et al., 2014), that microbial metabolites signal to the brain (Cryan et al., 2019), and that the cognitive benefit of TRE in patients with AD was attributed to a *B. pseudolongum*–propionic acid–FFAR3 pathway (Zhao et al., 2025). Figure 1 now shows this pathway, and the trial measures fecal short-chain fatty acids (Section 5.4 and Table 3). We also state the falsification logic: if cognition improves without glymphatic change, the result would favour these alternatives over the Dual-Gating model (Sections 3.4 and 5.7).

Although protein intake is mentioned, the proposed intervention may still reduce total energy intake or impair meal-based protein distribution in older adults. The trial design should specify monitoring of body composition, muscle strength, physical function, and adherence to adequate energy and protein intake.

Response 2.6: We agree. The trial design now includes weight-maintenance energy prescriptions with biweekly weight checks, individualized protein targets distributed across the meals in the window, and the same activity guidance across arms (Sections 5.2 and 5.3). It also monitors body composition (DXA at 0, 6, and 12 months), muscle strength (handgrip) and physical function (SPPB) every 3 months, and dietary adherence (24-hour recalls every 3 months). Frail and sarcopenic participants are excluded, and pre-specified stopping rules apply. The schedule is shown in Table 3. Please also see our Response 1.8.

REVIEWER 3

The paper proposes a hypothesis that assumes that early time-restricted eating is protective in neurodegenerative diseases. The authors propose 3 mechanisms by which eTRE protects against cognitive decline, as well as a three-arm randomized trial that could test the hypothesis.

The hypothesis is interesting, well documented, and the proposed mechanisms of action can be tested in a randomized study.

Response: We thank the Reviewer for the positive assessment and for the helpful suggestions, which we have addressed as follows.

I suggest the following corrections and additional explanations to the authors:

- in the abstract and main text, you repeat in several places that eTRE includes consuming at least 80% of daily calories before 19:00. In the randomized trial proposal, you suggest comparing eTRE with 80% of calories before 4:00 p.m., which is much more logical. I believe that in the abstract and in the text preceding the experiment proposal, you specified the wrong time, while in the experiment proposal you specified the correct time frame. Specifically, if a participant consumes 20% of their calories after 7:00 PM, it is very difficult to reconcile this with the requirement of not eating for at least three hours before bedtime; this automatically classifies the participant as having a late chronotype and shifts the eating time- window.

Response 3.1: We thank the Reviewer for this careful observation, and we agree. The 19:00 threshold came from an earlier, broader observational definition and was inconsistent with the trial arm. We have harmonized the definition throughout the Abstract, Section 3.1, and the trial. eTRE is now defined as (a) $\geq 80\%$ of daily energy consumed before 16:00, (b) an eating window of ≤ 10 hours starting after habitual wake time, and (c) ≥ 3 hours between the last intake and habitual sleep onset. With a 16:00 cut-off, criterion (c) is usually exceeded by a wide margin (about 6–7 hours). The eTRE arm is prescribed 08:00–16:00, and adherence is defined as $\geq 80\%$ of days with all intake inside the window. Section 3.1 also explains why the 19:00 cut-off was abandoned, following the Reviewer's reasoning: allowing up to 20% of energy after 19:00 is hard to reconcile with a long pre-sleep fast, and it does not clearly separate early from late eaters.

- Some parts of sentences or entire sentences are very difficult to follow. All such sentences are highlighted in yellow in the text; please try to simplify them or provide further explanation.

Response 3.2: We thank the Reviewer and apologize for these passages. We rewrote each of the six highlighted passages, as detailed below, and we also shortened other long sentences throughout the manuscript (all changes are visible in the tracked-changes version). We would be glad to revise any passage that the Reviewer still finds unclear.

(a) Abstract, description of the pathways. Original: “restoration of oscillatory suppression of pro-inflammatory cytokines through microglial BMAL1 and CLOCK stabilization; and preservation of hippocampal synaptic plasticity via rhythmic clock-gene regulation.” Revised: “We evaluate three candidate pathways: daily rhythms in the removal of amyloid-beta and tau from the brain (glymphatic clearance); daily rhythms that keep brain inflammation low; and clock genes that support memory-related plasticity in the hippocampus.”

(b) Abstract, Dual-Gating model. Original: “a ‘Dual-Gating’ model combining a pre-sleep autonomic gate with a nocturnal metabolic gate.” Revised: “An AI co-scientist analysis, checked by the authors, suggested a ‘Dual-Gating’ model: finishing meals early may lower nervous-system arousal and insulin levels at night, and both changes may favour brain clearance during sleep.”

(c) Abstract, trial and mediation analysis. Original: “with structural equation modeling required to confirm that any cognitive benefit is mediated by glymphatic and inflammatory biomarkers.” Revised: “We outline a three-arm trial (eTRE, late time-restricted eating, habitual eating) that tests whether any cognitive benefit is explained by changes in markers of clearance and inflammation.” The statistical method (structural equation modeling) is now explained in Section 5.7.

(d) Introduction, definition of chrononutrition. Original: “This absence is scientifically notable: chrononutrition, the field examining how the temporal patterning of food intake interacts with endogenous circadian clocks, provides a biologically coherent mechanism by which eating schedules could influence brain health independently of dietary composition.” Revised: “Meal timing is not on this list. Chrononutrition studies how the timing of food intake interacts with the body’s internal clocks. It offers a plausible route by which eating schedules could affect brain health, separately from what or how much people eat.”

(e) Implications, relation to sleep and physical activity. Original: “If eTRE acts on the same downstream mechanisms as sleep and physical activity but through a partially independent chronobiological route, dementia prevention gains a modifiable factor that is accessible, low-cost, and potentially scalable to populations.” Revised (Section 6): “Sleep and physical activity are thought to protect the brain partly through the same mechanisms discussed here. If eTRE acts on these mechanisms by a different route, namely the timing of meals, dementia prevention research would gain a new intervention to test. Such an intervention would be cheap, easy to adopt, and could be offered to large populations.”

(f) Implications, long-term care facilities. Original: “Long-term care facility residents present both a methodological challenge (individually directed eTRE protocols require institutional cooperation) and a scientific opportunity (standardized institutional meal schedules create natural experimental variation in circadian alignment amenable to observational study as a complement to controlled trials).” Revised (Section 6): “In long-term care facilities, meal times are set by the institution rather than by residents. Individual eTRE protocols would therefore be hard to deliver without the facility’s cooperation. On the other hand, meal schedules differ between facilities. Comparing residents of facilities with earlier and later meal times could provide observational evidence to complement controlled trials.”

- You state that you used AI co-scientist analysis to generate the hypothesis and model, yet you mention all AI tools in the acknowledgments. This is insufficient. You need a

methodological section clarifying how these tools were used, including the exact prompt that led to the generation of the model or hypothesis. Only then are you transparent about your methodology and able to demonstrate that the idea generated in this way is novel.

Response 3.3: We agree that transparency requires more than an acknowledgment. We added a new methodological section, Section 2 (“Hypothesis Development and Use of Generative AI”). It reports the tool (Google Hypothesis Generation, AI co-scientist), the date of the run (17 June 2026), the enabled data sources, and the process (generation, critique, tournament ranking, and refinement). In this system, the user’s prompt is recorded as the Research Summary of the run specification, and the system then structures it into focus areas and preferences before the run. The complete run specification, including the exact prompt (Research Summary), the focus areas, and the preferences, is reproduced verbatim in the new Supplementary File S1. File S1 also includes a table that states the origin of each element of the manuscript (human draft, authors’ prompt, or AI output) and how it was verified. Section 2.3 describes what the system contributed, which proposals were rejected, and how the authors verified the retained elements. This also allowed us to make the attribution more precise: the mediation requirement was part of the authors’ prompt, whereas the three-arm design was introduced by the system at the goal-specification stage and the Dual-Gating model came from the system’s generated output. The full AI output (knowledge base, summary report, and ranked idea reports) is available on request (Data Availability Statement). We also note that we do not claim novelty because of the AI process; the novelty claims rest on the literature (see Response 3.5).

- In the introduction, you state that 14 risk factors for neurodegeneration have been identified—factors you mention again and partially list in the implications chapter. Please list them at the very beginning so that your proposed risk factor can be placed in the context of all the others.

Response 3.4: Thank you for this suggestion. The first paragraph of the Introduction now lists all fourteen risk factors of the 2024 Lancet Commission: less education, hearing loss,

high LDL cholesterol, depression, traumatic brain injury, physical inactivity, diabetes, smoking, hypertension, obesity, excessive alcohol consumption, social isolation, air pollution, and untreated vision loss.

- The hypothesis you propose is not entirely new, as it was also addressed in the studies by Hatori et al. and Zhao et al. cited in your references; however, you offer a novel mechanistic explanation, propose a dual model, and suggest the addition of a new risk factor.

Response 3.5: We agree and thank the Reviewer. A new paragraph in the Introduction now acknowledges this prior work. It notes that TRF prevented metabolic disease in mice without reducing caloric intake (Hatori et al., 2012) and that TRF/TRE was associated with better cognition in patients with AD through a gut microbiota pathway (Zhao et al., 2025). It then states the three ways in which the present review adds to this work: it asks whether the circadian position of the eating window, and not only its length, matters; it proposes a specific and testable mechanistic model (the Dual-Gating model); and it describes a trial that can separate timing from fasting duration. The last row of Table 4 (“Balance between novelty and established paradigms”) was revised in the same way. Following comments from Reviewer 1, we also moderated the suggestion that meal timing could become a new risk factor (see Response 1.9).

- Cite the literature for this statement: Finally, compressing the feeding window in older adults raises a sarcopenic risk: adequate total protein intake (1.2–1.6 g/kg/day) and its distribution across meals, determinants of the anabolic response in aging muscle, must be monitored and maintained in any future trial.

adequate total protein intake (1.2–1.6 g/kg/day) and its distribution across meals, determine

nants of the anabolic response in aging muscle, must be monitored and maintained in any

future trial.

Response 3.6: We thank the Reviewer. This passage (now in Section 4.1) is now supported by references: protein requirements of older adults (Bauer et al., 2013, PROT-AGE Study Group; Deutz et al., 2014, ESPEN Expert Group), the effect of protein distribution across meals on muscle protein synthesis (Mamerow et al., 2014), and lean-mass loss in a TRE trial (Lowe et al., 2020). While checking these sources, we corrected the recommended intake to match them: 1.0–1.2 g/kg/day for healthy older adults and 1.2–1.5 g/kg/day for those with acute or chronic disease. The practical safeguards are now described in Section 5.2 (see Response 1.8).

- on what basis do you say that the minimum duration of a randomized study is 12 months? This is a long period and many subjects might drop out of the study.

Response 3.7: We thank the Reviewer for this question. A new Section 5.6 (“Trial Duration”) explains the choice. First, cognitive change in at-risk older adults is slow; for example, the FINGER multidomain trial used two years to detect cognitive effects (Ngandu et al., 2015). Second, plasma p-tau217 changes over years rather than months (Mattsson-Carlsson et al., 2020), so shorter trials would mainly detect metabolic effects. Third, the design needs an intermediate time point (6 months) to measure the mediators before the 12-month outcome. We agree that attrition is a real risk. We now (i) budget 25–30% attrition in the sample size (Section 5.7); (ii) add a 3-month feasibility pilot to estimate adherence and retention before the main trial; (iii) describe retention strategies (regular dietitian contact, flexible visits, and transport support); and (iv) pre-specify multiple imputation with sensitivity analyses for missing data.

-You did not specify how many times the biochemical, cognitive, and DTI-ALPS assessments would be performed during the study—only at the beginning and the end?

Response 3.8: Thank you for pointing out this omission. A new Table 3 (“Proposed assessment schedule”) specifies the timing of every assessment. Cognitive tests (PACC-5, MoCA) are done at 0, 6, and 12 months. Plasma p-tau217, p-tau181, and NfL are measured at 0, 3, 6, and 12 months. DTI-ALPS, with free-water and white-matter metrics and FLAIR/T2 imaging, is done at 0, 6, and 12 months. Diurnal cytokines, the cortisol awakening response, and metabolic and microbiota markers are measured at 0, 6, and 12 months. Actigraphy, sleep questionnaires, and dietary recalls are done every 3 months. DXA is done at 0, 6, and 12 months, and handgrip strength, SPPB, and body weight every 3 months (weight also every 2 weeks). Section 5.4 explains that the 6-month mediator measurements precede the 12-month primary cognitive endpoint, so the mediation analysis respects temporal order (Cole and Maxwell, 2003).