

Appendix Section B(2): CONCEPT PAPER TEMPLATE

**DUNEDIN MULTIDISCIPLINARY HEALTH AND  
DEVELOPMENT STUDY**  
**(The Dunedin Study)**

**CONCEPT PAPER TEMPLATE**  
**(July 2024)**



## DUNEDIN STUDY CONCEPT PAPER

**Provisional Paper Title:** Is early menopause preceded by accelerated aging and risk for Alzheimer's disease?

**Proposing Author:** Ethan Whitman

**Author's Email:** ethan.whitman@duke.edu

**P.I. Sponsor:** Ahmad Hariri, Terrie Moffitt, Avshalom Caspi  
(if the proposing author is a student or colleague of an original PI)

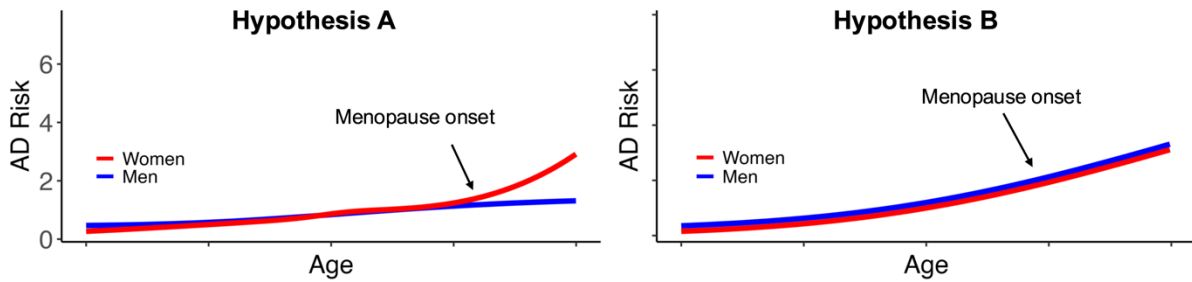
**Today's Date:** 2/25/25

---

Please describe your proposal in 2-3 pages with sufficient detail for helpful review by addressing all areas outlined below.

### **Objective of the study:**

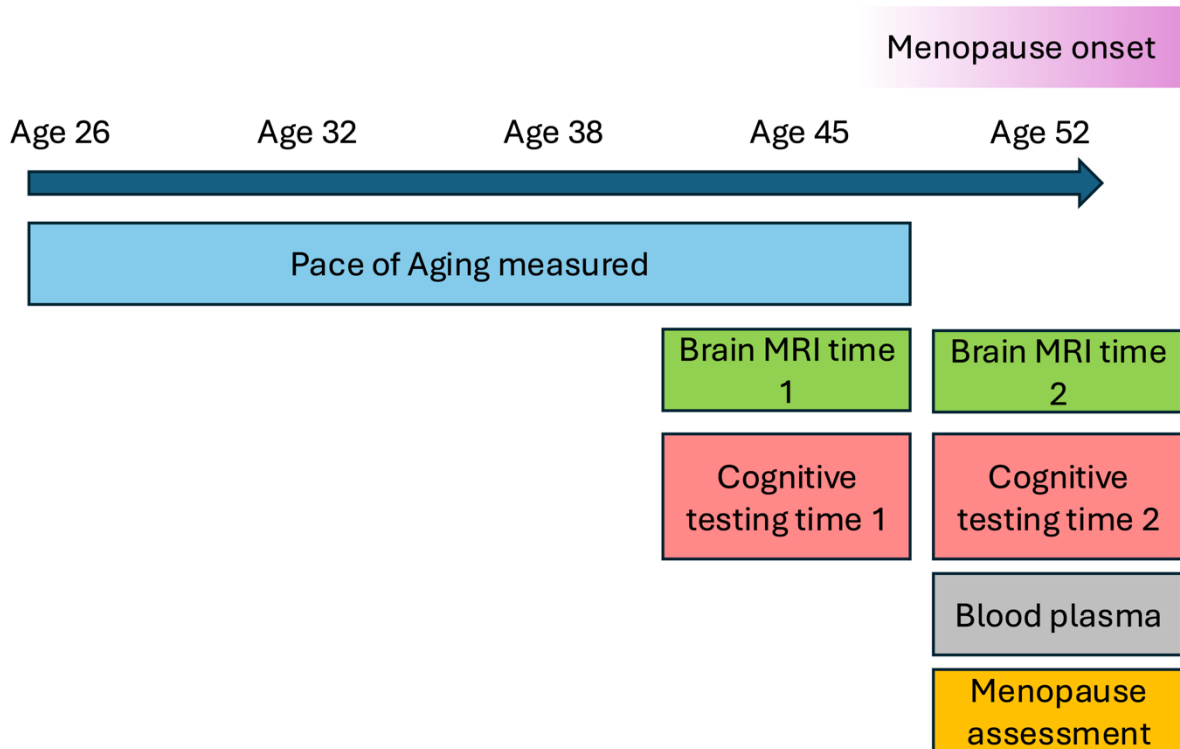
Earlier menopause is associated with higher risk for chronic aging-related diseases, including Alzheimer's disease (AD), as well as earlier all-cause mortality (Dobson et al., 2024; Ossewaarde et al., 2005). Further, there appears to be some evidence that menopause may cause aging to accelerate (Daredia et al., 2025; Levine et al., 2016; Lu et al., 2019; Mason et al., 2009). This has highlighted earlier age at menopause as a key risk factor for dementia (Dobson et al., 2024) and has identified hormone replacement therapy (HRT) as a promising intervention that may prevent AD (Nelsan et al., 2023). However, less is known about whether accelerated somatic aging may also precede the onset of earlier menopause. This is crucial to establish because accelerated somatic aging is also a risk factor for AD (Gampawar et al., 2025; Savin et al., 2024; Sugden et al., 2022), including specifically among women (Nguyen et al., 2024). Thus, earlier menopause could represent a dramatic inflection point in women's aging trajectories (Figure 1, Hypothesis A). On the other hand, it could also represent a continuation of already accelerated somatic aging (Figure 2, Hypothesis B). Determining the temporal order of earlier menopause and accelerated somatic aging would help identify the earliest points in the window of opportunity for neuroprotective interventions, such as HRT, against AD in women. Likewise, this will answer the degree to which neuroendocrine interventions should be preceded by or paired with general preventative interventions to further mitigate AD risk. Unfortunately, existing research has been unable to determine this temporal order and, subsequently, identify the full potential of the critical window of opportunity for early interventions. This is due, in part, to three key limitations.



**Figure 1. Competing hypothesis describing the temporal relationship between menopause onset and accelerated somatic aging.**

First, studies have typically collected relevant data decades after menopause has occurred (Daredia et al., 2025; Levine et al., 2016; Lu et al., 2019; Ossewaarde et al., 2005). While such studies allow for tests of long-term sequelae of earlier menopause, they preclude investigation of how premenopausal somatic aging might contribute to clinical outcomes. Second, studies comparing somatic aging across women who are pre-, peri-, or post-menopausal are nearly always confounded by differences in chronological age (Barth et al., 2024; Crestol et al., 2024). This is difficult to avoid, given the highly age-dependent trajectory of menopause. Nonetheless, it complicates the ability to draw conclusions about the relationship between menopause and accelerated somatic aging independently of chronological age. Third, studies linking menopause and common aging biomarkers are confounded by the correlation between menopause and chronological age (Daredia et al., 2025; Levine et al., 2016). Common aging biomarkers such as epigenetic clocks are designed to estimate chronological age in large samples of adults, including women who are pre-, peri-, and post-menopausal. Because the age range during which menopause occurs is relatively narrow (mean age = 50-52, SD = 4.5 years) (Appiah et al., 2021; Arinkan & Gunacti, 2021) it means that these clocks are, by definition, inadvertently trained to predict menopause status itself. Thus, reported association between menopause status and somatic aging biomarkers is somewhat circular.

We propose to overcome these key limitations by leveraging a unique existing dataset to identify the critical window for HRT to mitigate risk for AD. Specifically, we will analyze data collected through the longitudinal Dunedin Study, which has followed a large population representative birth cohort for six decades. The 1,037 members of the Dunedin Study have been repeatedly studied at ages 0, 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, 38, 45, and currently age 52 – the average year of menopause onset. The timing of relevant measures across waves of the Dunedin Study is presented in Figure 2. We will determine the temporal order of accelerated somatic aging and menopause onset (Aim 1). We will further examine how accelerated somatic aging and menopause may interact to shape sex-specific AD midlife risk, operationalized through cognitive and brain MRI measures (Aims 2-3).



**Figure 2. Timing and sequence of relevant measurements across waves of the Dunedin Study.**

### Data analysis methods<sup>1</sup>:

#### **Aim 1. Does accelerated somatic aging precede earlier menopause?**

We have operationalized somatic aging by longitudinally collecting 19 biomarkers of metabolic, cardiovascular, kidney, respiratory, immune, and dental organ system functioning at ages 26, 32, 38, and 45 years. Using linear mixed-effects models, we quantified the rate of change in these 19 biomarkers for each Study member. We combined this information across all 19 biomarkers to create a composite measure of somatic aging referred to as the Pace of Aging. We have used this longitudinal measure to demonstrate that accelerated somatic aging is associated with a broad range of outcomes that predict later AD risk including faster cognitive decline, poorer structural brain integrity, and diminished sensorimotor capacity (Elliott et al., 2021). Critically, as only two Study women reported menopause onset by age 45, confirmed by plasma-estrogen testing, the Pace of Aging is derived from data unbiased by effects of menopause. Furthermore, because each biomarker was standardized within men and women Study members, the Pace of Aging is independent of sex-specific trends in biomarker change that might bias associations with menopause.

<sup>1</sup> A key concern for the Dunedin Study is superficial analyses of data that simply identify differences or deficits between ethnic groups or other communities where inequities exist (e.g. persons with disabilities, Pasifika peoples, members of migrant and SOGIESC (Sexual Orientation, Gender Identify and Expression and Sexual Characteristics) communities). The cumulative effect of these types of studies is stigmatising and not of benefit. Any research that identifies differences must (a) incorporate information on the broader context (e.g. historical or political factors); (b) where possible undertake additional analyses to examine the source of the difference/s, and (c) include policy recommendations for its resolution.

We will use self-reported and plasma-determined estrogen levels at age 52 to classify Study members into three groups: postmenopausal, perimenopausal, and premenopausal. Because all Study members are the same chronological age, the postmenopausal group (projected N=150) represents earlier-than-average menopause, the perimenopausal group (projected N=150) represents average age at menopause, and the premenopausal group (projected N=150) represents later-than-average menopause.

We will then test whether somatic aging, as measured by the Pace of Aging from ages 26-45, varies as a function of menopause status at age 52. Of note, it is not necessary to control for sex, age, or HRT in this analysis as all Study members are the same age and sex, and no Study members were undergoing HRT prior to age 45. This analysis will allow us to test whether earlier menopause is preceded by accelerated somatic aging as assessed up to age 45. If accelerated aging precedes earlier menopause, this would suggest that interventions to slow somatic aging should begin prior to the onset of menopause and, possibly, be considered alongside neuroendocrine interventions in post-menopausal women. If not, it would suggest that AD prevention should prioritize interventions in post-menopausal women, potentially through interventions such as HRT.

### **Aim 2. Does earlier menopause precede accelerating midlife AD risk independently of prior somatic aging?**

We will test whether menopause status is associated with change in cognitive, brain MRI from age 45 to 52, as well as levels of AD blood plasma biomarkers collected at age 52. We have previously collected cognitive assessments, and brain MRI from Study members at age 45 and are currently collecting these measures again at age 52. Our cognitive measures include a wide battery of cognitive measures, including of age-sensitive domains such as memory and processing speed. Our brain MRI data allows us to measure features of structural brain integrity that are associated with later AD risk and clinical progression including cortical thickness, hippocampal volume, burden of white matter hyperintensities. Finally, we plan to assay  $A\beta_{42}/A\beta_{40}$  ratio, P-tau<sub>181</sub>, and neurofilament light chain (NfL) from blood plasma collected at age 52.

These data will allow us to first test whether earlier menopause is linked to more rapid changes in midlife measures of AD risk. We will test for associations between pre-, peri-, or post-menopause status and change in cognition and brain structure between age 45 and 52. In addition, we will test for associations between menopause status and levels of plasma AD biomarkers at age 52. Comparison of these groups will allow us to gauge whether earlier menopause occurs alongside more rapid cognitive decline, loss of brain structural integrity, or higher levels of AD pathology. Crucially, we will be able to compare Study women of the same age who did or did not experience menopause by age 52 – allowing for a powerful control group for associations between earlier menopause and change in AD risk.

Next, we will ask whether any associations between menopause and AD risk markers are accounted for by accelerated somatic aging (i.e. the Pace of Aging between ages 26 and 45). Again, it is not necessary to control for sex or age in this analysis; however, we will control for hormone replacement therapy at age 52. We will also control for broader AD risk in midlife using a multimodal composite index including genetic, health, and environmental factors (Reuben et al., 2022) derived at age 45. These analyses will help establish if earlier menopause initiates additional AD risk in midlife above and beyond general somatic aging. If so, this would suggest menopause is an inflection point in the progression of AD risk and that the onset of menopause represents the beginning of a critical window for intervention, potentially through HRT.

### **Aim 3. Does earlier menopause interact with somatic aging to shape sex-specific AD risk?**

We will compare midlife AD risk biomarkers in post-, peri-, and premenopausal women to those in men to establish whether menopause interacts with somatic aging to shape sex-specific AD risk. Specifically, we will create control groups of men matched with each menopausal group on the Pace of Aging. Next, we will test for differences in the midlife AD risk biomarkers from Aim 2 between each menopausal group and their respective male control group. Again, it is not necessary to control for age in this analysis; however, we will control for HRT at age 52. These analyses will help link earlier menopause to sex differences in AD prevalence and clinical progression (“2022 Alzheimer’s disease facts and figures,” 2022). If postmenopausal women, but not premenopausal women, show increased burden of midlife AD risk compared to men with comparable somatic aging, this suggests that earlier menopause may initiate sex-specific neuroendocrine changes that may ultimately predispose women to the amyloid cascade, neurodegeneration, and AD. This would further highlight menopause as the initiation of a critical window for intervention and suggest that neuroendocrine interventions such as HRT are most likely to be effective.

#### **Variables needed at which ages:**

Sex

#### **Phase 45:**

Pace of Aging, **PaceOfAgingP45**

Age 45 IQ, **IQ45\_chstd**

Cortical thickness, **img\_CT\_AVG45**

Cortical surface area, **img\_SA\_TOT45**

Ventricular volume

Subcortical volumes (accumbens, amygdala, caudate, cerebellum, hippocampus, pallidum, putamen, thalamus, ventral DC, brainstem)

DunedinARB index, **ARD\_DunedinARB45**

#### **Phase 52:**

Self-reported menopause status  
 Estrogen plasma levels  
 HRT medication status  
 Age 52 IQ  
 Cortical thickness  
 Cortical surface area  
 Ventricular volume  
 Subcortical volumes (accumbens, amygdala, caudate, cerebellum, hippocampus, pallidum, putamen, thalamus, ventral DC, brainstem)  
 A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> ratio from blood plasma  
 P-tau<sub>181</sub> from blood plasma  
 neurofilament light chain (NfL) from blood plasma

### **Significance of the Study (for theory, research methods or clinical practice):**

Establishing the temporal order of accelerated somatic aging and earlier menopause is crucial for understanding the role of menopause in AD risk and targeting early interventions to maximize effectiveness. Our proposed analyses will leverage unique data from an ongoing longitudinal study to help establish whether the 'window of opportunity' for intervention begins prior to menopause, and whether neuroendocrine interventions such as HRT should be preceded by or paired with general anti-aging interventions to maximize effectiveness among women aging more quickly.

### **How the paper will contribute to Māori health advancement and/or equitable health outcomes<sup>2</sup>**

This study may have implications for sex-specific disease risk factors and women's health. Women's health, particularly in neuroscience, is badly understudied (Jacobs, 2023). Thus, this work may help improve our understanding of a major female-specific component of aging. This study will not include separate analysis of specific ethnic groups, but the results are expected to be generalizable to the Māori community. Accelerated aging and dementia risk are thought to be more pronounced in disadvantaged communities and an improved understanding on how menopause is related to aging and dementia will help support those most at risk.

### **References:**

- 2022 Alzheimer's disease facts and figures. (2022). *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 18(4), 700–789.
- Appiah, D., Nwabuo, C. C., Ebong, I. A., Wellons, M. F., & Winters, S. J. (2021). Trends in age at natural menopause and reproductive life span among US women, 1959-2018. *JAMA: The Journal of the American Medical Association*, 325(13), 1328–1330.

<sup>2</sup> Helpful information can be found here: [https://www.hrc.govt.nz/sites/default/files/2020-01/NZ%20Prioritisation-Framework-FA-web\\_0.pdf](https://www.hrc.govt.nz/sites/default/files/2020-01/NZ%20Prioritisation-Framework-FA-web_0.pdf)

- Arinkan, S. A., & Gunacti, M. (2021). Factors influencing age at natural menopause. *The Journal of Obstetrics and Gynaecology Research*, 47(3), 913–920.
- Barth, C., Galea, L. A. M., Jacobs, E. G., Lee, B. H., Westlye, L. T., & de Lange, A.-M. G. (2024). Menopausal hormone therapy and the female brain: leveraging neuroimaging and prescription registry data from the UK Biobank cohort. <https://doi.org/10.7554/elife.99538>
- Belsky, D. W., Caspi, A., Corcoran, D. L., Sugden, K., Poulton, R., Arseneault, L., Baccarelli, A., Chamarti, K., Gao, X., Hannon, E., Harrington, H. L., Houts, R., Kothari, M., Kwon, D., Mill, J., Schwartz, J., Vokonas, P., Wang, C., Williams, B. S., & Moffitt, T. E. (2022). DunedinPACE, a DNA methylation biomarker of the pace of aging. *ELife*, 11. <https://doi.org/10.7554/eLife.73420>
- Crestol, A., de Lange, A.-M. G., Schindler, L., Subramaniapillai, S., Nerland, S., Oppenheimer, H., Westlye, L. T., Andreassen, O. A., Agartz, I., Tamnes, C. K., & Barth, C. (2024). Linking menopause-related factors, history of depression, APOE  $\epsilon$ 4, and proxies of biological aging in the UK biobank cohort. *Hormones and Behavior*, 164(105596), 105596.
- Daredia, S., Khodasevich, D., Gladish, N., Shen, H., Nwanaji-Enwerem, J. C., Bozack, A. K., Needham, B. L., Rehkopf, D. H., Deardorff, J., & Cardenas, A. (2025). Timing of menarche and menopause and epigenetic aging among U.S. adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Clinical Epigenetics*, 17(1), 31.
- Dobson, A. J., Xu, Z., Wilson, L. F., Chung, H.-F., Sandin, S., Van der Schouw, Y. T., Demakakos, P., Weiderpass, E., & Mishra, G. D. (2024). Menopause age and type and dementia risk: a pooled analysis of 233 802 women. *Age and Ageing*, 53(11). <https://doi.org/10.1093/ageing/afae254>
- Elliott, M. L., Caspi, A., Houts, R. M., Ambler, A., Broadbent, J. M., Hancox, R. J., Harrington, H., Hogan, S., Keenan, R., Knodt, A., Leung, J. H., Melzer, T. R., Purdy, S. C., Ramrakha, S., Richmond-Rakerd, L. S., Righarts, A., Sugden, K., Thomson, W. M., Thorne, P. R., ... Moffitt, T. E. (2021). Disparities in the pace of biological aging among midlife adults of the same chronological age have implications for future frailty risk and policy. *Nature Aging*, 1(3), 295–308.
- Gampawar, P., Veeranki, S. P. K., Petrovic, K.-E., Schmidt, R., & Schmidt, H. (2025). Epigenetic age acceleration is related to cognitive decline in the elderly: Results of the Austrian Stroke Prevention Study. *Psychiatry and Clinical Neurosciences*. <https://doi.org/10.1111/pcn.13793>
- Jacobs, E. G. (2023). Only 0.5% of neuroscience studies look at women's health. Here's how to change that. *Nature*, 623(7988), 667.
- Levine, M. E., Lu, A. T., Chen, B. H., Hernandez, D. G., Singleton, A. B., Ferrucci, L., Bandinelli, S., Salfati, E., Manson, J. E., Quach, A., Kusters, C. D. J., Kuh, D., Wong, A., Teschendorff, A. E., Widschwendter, M., Ritz, B. R., Absher, D., Assimes, T. L., & Horvath, S. (2016). Menopause accelerates biological aging. *Proceedings of the National Academy of Sciences of the United States of America*, 113(33), 9327–9332.
- Lu, A. T., Quach, A., Wilson, J. G., Reiner, A. P., Aviv, A., Raj, K., Hou, L., Baccarelli, A. A., Li, Y., Stewart, J. D., Whitsel, E. A., Assimes, T. L., Ferrucci, L., & Horvath, S. (2019). DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging*, 11(2), 303–327.

- Mason, J. B., Cargill, S. L., Anderson, G. B., & Carey, J. R. (2009). Transplantation of young ovaries to old mice increased life span in transplant recipients. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 64(12), 1207–1211.
- Nelson, L. S., Mørch, E. A., Holm, C., & Torp-Pedersen, A. (2023). Menopausal Hormone Therapy and Dementia: Nationwide, Nested Case-Control Study. *Clinical Research Ed.*, 381.
- Nguyen, S., McEvoy, L. K., Espeland, M. A., Whitsel, E. A., Lu, A., Horvath, S., Manson, J. E., Rapp, S. R., & Shadyab, A. H. (2024). Associations of epigenetic age estimators with cognitive function trajectories in the Women's Health Initiative Memory Study. *Neurology*, 103(1), e209534.
- Ossewaarde, M. E., Bots, M. L., Verbeek, A. L. M., Peeters, P. H. M., van der Graaf, Y., Grobbee, D. E., & van der Schouw, Y. T. (2005). Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology (Cambridge, Mass.)*, 16(4), 556–562.
- Reuben, A., Moffitt, T. E., Abraham, W. C., Ambler, A., Elliott, M. L., Hariri, A. R., Harrington, H., Hogan, S., Houts, R. M., Ireland, D., Knodt, A. R., Leung, J., Pearson, A., Poulton, R., Purdy, S. C., Ramrakha, S., Rasmussen, L. J. H., Sugden, K., Thorne, P. R., ... Caspi, A. (2022). Improving risk indexes for Alzheimer's disease and related dementias for use in midlife. *Brain Communications*, 4(5), fcac223.
- Savin, M. J., Wang, H., Pei, H., Aiello, A. E., Assuras, S., Caspi, A., Moffitt, T. E., Muenning, P. A., Ryan, C. P., Shi, B., Stern, Y., Sugden, K., Valeri, L., & Belsky, D. W. (2024). Association of a pace of aging epigenetic clock with rate of cognitive decline in the Framingham Heart Study Offspring Cohort. *Alzheimer's & Dementia (Amsterdam, Netherlands)*, 16(4), e70038.
- Sugden, K., Caspi, A., Elliott, M. L., Bourassa, K. J., Chamarti, K., Corcoran, D. L., Hariri, A. R., Houts, R. M., Kothari, M., Kritchevsky, S., Kuchel, G. A., Mill, J. S., Williams, B. S., Belsky, D. W., Moffitt, T. E., & Alzheimer's Disease Neuroimaging Initiative\*. (2022). Association of pace of aging measured by blood-based DNA methylation with age-related cognitive impairment and dementia. *Neurology*, 99(13), e1402–e1413.
- Whitman, E. T., Elliott, M. L., Knodt, A. R., Abraham, W. C., Anderson, T. J., Cutfield, N., Hogan, S., Ireland, D., Melzer, T. R., Ramrakha, S., Sugden, K., Theodore, R., Williams, B. S., Caspi, A., Moffitt, T. E., Hariri, A. R., & Alzheimer's Disease Neuroimaging Initiative. (2024). An estimate of the longitudinal pace of aging from a single brain scan predicts dementia conversion, morbidity, and mortality. In *bioRxiv*. <https://doi.org/10.1101/2024.08.19.608305>