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Research Article

Associations Between Loneliness, Epigenetic Aging, and Multimorbidity Through Older Adulthood

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Abstract

Objectives: Loneliness is a pressing public health concern, but the mechanisms by which it leads to declining physical health are uncertain. Prior work has begun to explore epigenetic pathways, with some evidence suggesting a link between loneliness and DNA methylation, though it is unclear whether epigenetic variation can help explain loneliness–health associations.

Methods: Associations between loneliness and epigenetic age acceleration (EAA) were estimated, as well as the degree to which EAA mediated and moderated the association between loneliness and the development of chronic physical health conditions (multimorbidity) in older adulthood. The sample consisted of Health and Retirement Study participants who provided blood draws and consented to methylation profiling (n = 4,018).

Results: Baseline loneliness was associated with greater EAA in the GrimAge measure net of demographic and behavioral covariates (β = 0.07, p = .003). Loneliness and GrimAge each predicted increasing condition counts, but there was no evidence of an interactive effect. The association between loneliness and increasing condition counts was, however, significantly mediated by GrimAge (indirect path β = 0.020, p = .003).

Discussion: These results suggest that the impact of loneliness on multimorbidity may, in part, operate through DNA methylation. The specific intermediary, physiological mechanisms that are involved will require further research, but EAA measures like GrimAge are promising in helping to understand the health impacts of loneliness.

Keywords: Biological embedding, Epigenetic age acceleration, Epigenetic clock, GrimAge, Methylation

Loneliness has been defined as the distressing emotional experience resulting from the perception that one's social needs are not being met by their relationships (Hawkley & Cacioppo, 2010). Loneliness, therefore, is usually operationalized with self-report measures, which are linked with a range of adverse physical health outcomes, including multimorbidity (Hajek et al., 2020) and early mortality (Wang et al., 2023). Loneliness's rising prevalence and strong associations with health have led to its increasing recognition as a public health epidemic, a designation underscored by the U.S. Surgeon General (O'Sullivan et al., 2022; Office of the Surgeon General (OSG), 2023). The presence of two or more chronic physical health conditions, or multimorbidity, is prevalent in older adulthood and linked with quality of life, disability, and mortality (Marengoni et al., 2011). Its generalized nature also makes multimorbidity helpful in understanding loneliness, likely capturing some of its impacts across multiple physiological systems (e.g., inflammatory, cognitive, metabolic). Associations between loneliness and health are partially attributable to behaviors like low physical activity and smoking (Luo & Waite, 2014; Patterson & Veenstra, 2010) or other influences that occur earlier in a causal chain (e.g., childhood rearing environment, socioeconomic factors), overlapping genetic variants (Abdellaoui et al., 2019), and reverse causation (i.e., poor health influencing social functioning; Holt-Lunstad et al., 2015). However, these factors likely cannot fully explain the relationship, consistent with loneliness having a causal impact on health (Freilich, 2023; Luo et al., 2012).

Hawkley and Cacioppo (2010) theorized that, because social connection is evolutionarily vital to safety, experiencing loneliness operates as an environmental threat that can set off hypervigilant physiological responses. Chronic responses of this nature may cause health declines, likely through pro-inflammatory processes involving increased production of cytokines and glucocorticoids and chronic activation of the hypothalamic–pituitary–adrenal (HPA) axis. Indeed, loneliness has been linked to variation in cortisol level and diurnal rhythm (Doane et al., 2013; Lai et al., 2019), as well as elevations in sympathetic nervous system (SNS) neuroeffector molecules like norepinephrine (Capitanio et al., 2019). Oxidative stress is likely an important molecular mechanism linking loneliness to the activation of stress response systems

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like the SNS and HPA axis (Li & Xia, 2020), which, in turn, can impact pathogenesis by altering gene transcriptional processes involved in pro-inflammatory signaling (Eachus & Cunliffe, 2018). Across several studies, loneliness has been linked with elevated pro-inflammatory gene expression and reduced antiviral expression (Cole, 2014; Cole et al., 2007), suggesting that its health impacts may operate through gene regulatory or epigenetic pathways.

DNA methylation is one such epigenetic process by which transcription is regulated. Variability in methylation levels across some regions of the genome relates strongly with age, allowing for the calculation of "epigenetic clocks," which use individuals' methylation profiles to estimate biological age as distinct from chronological age. Various algorithms have been constructed to estimate biological or epigenetic age, with "second-generation" measures designed to optimally predict phenotypic characteristics of aging such as disease incidence, longevity, or disability, in contrast to "first-generation" measures developed to exclusively predict chronological age. To the extent that an individual's epigenetic age exceeds their chronological age, they are said to show "epigenetic age acceleration" (EAA). Second-generation EAA measures like GrimAge (Lu et al., 2019) and DunedinPoAm38 (Belsky et al., 2020) predict many adverse physical health outcomes, including all-cause mortality net of traditional risk factors (Faul et al., 2023; Oblak et al., 2021).

Supporting the theory that epigenetic processes contribute to the biological embedding of psychosocial environmental adversities (Eachus & Cunliffe, 2018), laboratory-induced psychosocial stress (Unternaehrer et al., 2012), and self-reported stress (Duman & Canli, 2015) have also been linked with methylation variability. As for loneliness, associations have also been observed with elevations in DunedinPACE (a newer version of DunedinPoAm38) and GrimAge (Beach et al., 2022; Freilich et al., 2024). Further, EAA is also associated with other social and relational variables, like attachment styles (Allen et al., 2022), social contact and support (Hillman et al., 2023), as well as volunteering status (Nakamura et al., 2023).

This initial evidence suggests modest links with various psychosocial stressors, though limited work has evaluated whether EAA can help explain associations between loneliness and adverse health outcomes. In an unpublished dissertation, Phillips (2020) found that methylation at specific CpG sites partially mediated the observed nonsignificant association between loneliness and longitudinal declines in processing speed, an important indicator of cognitive health. Similarly, Lynch et al. (2023) presented results consistent with an indirect effect of certain trajectories of loneliness on future cognitive ability mediated through GrimAge. In both studies, indirect effects were modest, especially when behavioral covariates were included in the models. Finally, Freilich et al. (2024) showed that DunedinPACE moderated midlife associations between loneliness and multimorbidity, such that loneliness' prediction of increases in chronic condition counts was more pronounced for individuals with greater EAA, while, in separate analyses, EAA was not a significant mediator of the association.

More work is needed to understand the pathways linking loneliness, EAA, and health outcomes like multimorbidity. EAA could plausibly mediate loneliness–disease associations, consistent with loneliness "getting under the skin" to affect health via DNA methylation. It is also plausible that methylation might affect biological responses to environmental risk factors and thereby alter vulnerability to loneliness–disease associations, consistent with moderation. Given the plausibility of both and the paucity of prior research, we did not make a priori hypotheses about which model might better fit the data. Rather, we aimed to replicate Freilich et al.'s (2024) midlife results in a larger and older sample and extend them by considering longitudinal change in both loneliness and chronic conditions. Thus, we sought to (a) quantify associations between initial levels of and change in loneliness with several EAA variables in older adulthood (see Author Note 1); (b) quantify associations between EAA and loneliness level and change with chronic health condition change; (c) test whether EAA might plausibly mediate longitudinal relationships between loneliness and chronic conditions; and (d) test whether EAA might moderate longitudinal relationships between loneliness and chronic conditions.

Method

Participants

The Health and Retirement Study (HRS) has a longitudinal panel design and surveys a representative sample of aging, American adults (Juster & Suzman, 1995). The analytic sample consisted of the subset of HRS participants who consented to DNA methylation assays as part of the 2016 Venous Blood Study (n = 4,018; Crimmins et al., 2017; Health and Retirement Study, 2024). We also used data from the HRS core survey to index demographic, psychosocial, and health factors. The 2016 survey sample was 58.5% female, and most participants identified as either White/Caucasian (75.0%) or Black/African American (16.8%). Descriptive statistics for all observed variables are reported in Supplementary Table 1.

Measures

Epigenetic age acceleration

DNA methylation assays were completed on a representative subsample of Venous Blood Study participants. Blood draws were intended to be scheduled within 4 weeks of the HRS core interview, so demographic information from the 2016 survey was treated as concurrent. After collection, tubes were shipped overnight to the Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing was done within 24 hr of arrival at the lab. DNA extracted from EDTA tubes was subject to methylation assays using the Infinium Methylation EPIC BeadChip. A total of 4,018 samples (97.9%) passed quality control procedures. Then, methylation profiles were scored using previously published algorithms to compute several measures of epigenetic age, including the first-generation Hannum (Hannum et al., 2013) and Horvath clocks (Horvath, 2013), the second-generation PhenoAge (Levine et al., 2018) and GrimAge (Lu et al., 2019) clocks, and the DunedinPoAm38 measure of EAA (Belsky et al., 2020). For more information on methylation data collection and processing, refer HRS documentation (https://hrs-data.isr.umich.edu/data-products/epigenetic-clocks).

While the first four algorithms produce estimates of epigenetic age in years, DunedinPoAm38 estimates the relative pace of recent aging as a multiplicative factor (i.e., a measure of age acceleration). Correspondingly, the other four clocks correlated strongly ($r \geq 0.73$) with chronological age, while DunedinPoAm38 had a minimal correlation ($r = 0.02$). To calculate EAA, the effect of chronological age was regressed

out of the four clocks, and the residuals were carried forward (i.e., epigenetic age net of chronological age). The resulting five EAA indices correlated weakly to moderately with one another ($0.09 \leq r \leq 0.64$), with the strongest associations observed between the later-generation GrimAge acceleration and DunedinPoAm38 measures ($r = 0.64$) and the earlier-generation Hannum and Horvath acceleration measures ($r = 0.44$). In addition, to capture shared variance among the measures, we calculated an "EAA average" composite by taking the arithmetic mean of the five other measures after standardization. Correlations between the EAA variables and other focal variables are reported in Supplementary Table 3.

Loneliness

An 11-item version of the UCLA Loneliness Scale (Russel, 1996) was administered to participants every 4 years such that approximately half the sample completed the items in 2010, 2014, and 2018, while the other half completed the items in 2008, 2012, and 2016. Participants were asked "How much of the time feel …" with items including "isolated from others," "alone," and "part of a group of friends" on a three-point scale (1 = Often; 2 = Some of the time; 3 = Hardly ever or never). After four items were reverse-scored, sum scores were calculated to index loneliness. Individual item means were imputed for missingness in participants who completed at least half of the items (see Author Note 2). Across the six waves, descriptive statistics and internal consistency estimates were similar; means ranged from 16.5 to 17.0, standard deviations ranged from 4.67 to 4.97, Cronbach's alphas ranged from 0.88 to 0.90, McDonald's omegas total ranged from 0.91 to 0.93, and McDonald's omegas hierarchical range from 0.70 to 0.74. To take advantage of all observations, we collapsed the data into three waves. The first wave consisted of the 2008 and 2010 administration of the UCLA scale ($n = 2{,}823$, $M = 16.55$), while the second collapsed the 2012 and 2014 data ($n = 3{,}362$, $M = 16.84$), and the third collapsed the 2016 and 2018 data ($n = 3{,}016$, $M = 16.94$). Of the entire analytic sample ($n = 4{,}018$), 2,152 had loneliness data across all three waves, 1,142 had data across two waves, 461 had data in just one wave, and 263 did not have loneliness data (see Author Note 3).

Chronic health conditions (multimorbidity)

The core survey sample was asked to report on 26 chronic health conditions and symptoms (e.g., stroke, hypertension, arthritis, shingles, chronic pain) in 2012 ($n = 3{,}933$), 2016 ($n = 4{,}018$), and 2020 ($n = 3{,}243$). For certain items, participants were asked if a doctor has ever told them they have the condition in the context of their previous survey administration (e.g., diabetes), and, for others, if they ever had the listed "persistent or troublesome problems" (e.g., severe fatigue, persistent cough/wheeze). Number of chronic conditions was indexed by counting the number of items endorsed (unit weighting). This variable was used to understand the construct of multimorbidity, so both terms are used moving forward, despite the range of the variable including zero and one (where multimorbidity is defined by the presence of two or more conditions). As expected, the average number of conditions endorsed increased from 2012 ($M_{conditions} = 4.29$, $M_{age} = 65.5$) to 2016 ($M_{conditions} = 4.97$, $M_{age} = 69.4$) to 2020 ($M_{conditions} = 5.12$, $M_{age} = 72.3$). A list of all conditions included and their frequencies in 2016 is given in Supplementary Table 2. Several of the conditions were cardiovascular and/or inflammatory in nature, which is notable given the evidence of the roles of oxidative stress, the HPA axis, and SNS in the theoretical pathways linking loneliness and health decline.

Covariates

In all models, the focal dependent variable was simultaneously regressed on the following demographic covariates: gender, chronological age, race, and years of education. With limited observations in other groups, race was coded with two binary variables corresponding to self-reported Black/African American (1 = Black [17% of sample], 0 = other) and White/Caucasian (1 = White [75%], 0 = other) racial identity. Chronological age was included in all models (even when the EAA outcome had already been regressed on age) given the risk of bias introduced by residual confounding when it is excluded (Krieger et al., 2023). Various health behaviors, in addition to demographics, were included as covariates in the next set of models. Self-reported number of cigarettes per day was included to control for the effects of smoking. We accounted for alcohol use with items related to frequency (days per week) and volume (average number per occasion) of drinking. Finally, body mass index (BMI) was included based on self-reports of height and weight. Most of the sample reported their height concurrent with the methylation blood draw (2016; 48.8%) or 2 years prior (2014; 49.7%) which allowed for concurrent (or near concurrent) measurement of BMI. We used self-reports from either 2012 or 2010 for the remaining participants.

Descriptive statistics for all covariates are reported in Supplementary Table 1 and correlations between the principal variables are reported in Supplementary Table 3. We report results from the models that include all covariates as primary (Tables 1 and 2) and results with only demographic covariates as Supplementary Tables 4 and 5.

Statistical Analysis

The analytic plan was to model initial levels and change in loneliness from 2008 to 2018 (collapsed between 2008–2012–2016 and 2010–2014–2018 for halves of the sample) as predictors of EAA (measured in 2016), and subsequently use both as predictors of condition count change (2012–2016–2020). Three sets of models were run across six different EAA variables and two different covariate sets (36 models in total) in Mplus 8.9 (Muthén & Muthén, 2024). The first set of regression models quantified associations between changes in loneliness with EAA, using linear growth modeling to operationalize intercept and slope terms for loneliness across three waves. We modeled freely correlating latent intercept and slope terms along a study measurement occasion time metric. The latent loneliness intercept and slope terms were simultaneously used as predictors of EAA in the broader structural equation model, along with the specified covariate set. To prevent list-wise deletion of observations with missingness, we specified distributional assumptions for covariates by modeling paths between pairs with nontrivial associations (e.g., drinking frequency and volume; age and BMI) across all models.

The second set of models tested whether EAA mediated the association between loneliness and multimorbidity. Condition count level and change were similarly modeled linearly across three waves. Given the importance of the development of multimorbidity through older adulthood, the condition count slope term was interpreted as the model's focal dependent

Table 1. Prediction of Epigenetic Age Acceleration by Latent Loneliness Level and Change

Predictor	Hannum acceleration $R^2 = 0.051$		Horvath acceleration $R^2 = 0.012$		PhenoAge acceleration $R^2 = 0.027$		GrimAge acceleration $R^2 = 0.316$		DunedinPoAm38 $R^2 = 0.161$		EAA average $R^2 = 0.153$	
	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value
Loneliness intercept	0.04 (0.02)	.08	0.03 (0.03)	.29	0.03 (0.03)	.19	**0.07 (0.02)**	**.003**	0.04 (0.03)	.13	**0.06 (0.03)**	**.01**
Loneliness slope	0.04 (0.04)	.35	−0.03 (0.04)	.49	−0.00 (0.04)	.96	0.08 (0.04)	.04	0.02 (0.04)	.60	0.03 (0.04)	.42
Chronological age	0.01 (0.02)	.64	0.03 (0.03)	.18	0.05 (0.02)	.04	**0.09 (0.02)**	**<.001**	**0.13 (0.02)**	**<.001**	**0.10 (0.02)**	**<.001**
Gender (1 = female, 0 = male)	**−0.17 (0.02)**	**<.001**	**−0.07 (0.02)**	**.001**	**−0.07 (0.02)**	**.001**	**−0.32 (0.02)**	**<.001**	**−0.07 (0.02)**	**<.001**	**−0.21 (0.02)**	**<.001**
Race (1 = Black, 0 = Other)	**−0.11 (0.02)**	**<.001**	−0.00 (0.02)	.94	0.02 (0.03)	.49	**0.11 (0.02)**	**<.001**	**0.06 (0.03)**	**.02**	0.02 (0.02)	.33
Race (1 = White, 0 = Other)	−0.01 (0.02)	.78	−0.00 (0.02)	.93	−0.02 (0.03)	.56	0.01 (0.02)	.60	−0.04 (0.03)	.15	−0.02 (0.02)	.50
School years	−0.02 (0.02)	.35	0.04 (0.02)	.07	−0.04 (0.02)	.08	**−0.14 (0.02)**	**<.001**	**−0.08 (0.02)**	**<.001**	**−0.07 (0.02)**	**<.001**
Smoking volume	**0.06 (0.02)**	**<.001**	0.03 (0.02)	.15	**0.06 (0.02)**	**.004**	**0.39 (0.03**	**<.001**	**0.34 (0.02)**	**<.001**	**0.27 (0.02)**	**<.001**
Avg # drinks	−0.01 (0.02)	.65	0.03 (0.02)	.12	**0.05 (0.02)**	**.03**	**0.08 (0.02)**	**.001**	**0.06 (0.02)**	**.006**	**0.06 (0.02)**	**.002**
Alcohol frequency	0.01 (0.02)	.83	−0.03 (0.02)	.24	0.02 (0.02	.42	−0.01 (0.02)	.53	−0.05 (0.02)	.03	−0.02 (0.02)	.40
BMI	**0.07 (0.02)**	**.002**	0.04 (0.02)	.11	**0.10 (0.02)**	**<.001**	**0.09 (0.02)**	**<.001**	**0.11 (0.02)**	**<.001**	**0.12 (0.02)**	**<.001**

Notes: Results that are statistically significant after accounting for the false discovery rate are printed in bold, given six comparisons and α = 0.05. Loneliness was measured in 2010, 2014, and 2018 for half the sample and 2008, 2012, and 2016 for the other half and operationalized as a linear growth curve across three timepoints (2008/10, 2012/14, and 2016/18). Gender was self-reported as a binary variable (1 = female; 0 = male). Given small samples across groups, race was coded with two binary variables. Number of years of education (*School years*) was self-reported. *Smoking volume* is operationalized as number of cigarettes per day (0 for nonsmokers). *Avg # drinks* was self-reported by asking participants about their typical amount of alcohol consumption when they drink. *Alcohol frequency* was self-reported by asking participants, "In the last three months, on average, how many days per week have you had any alcohol to drink?" (0–7). BMI was calculated using participant's self-reported heights and weights. Root mean square error of approximation (RMSEA) ranged from 0.035 to 0.036 across models. β = standardized multiple regression coefficient; BMI = body mass index; EAA = epigenetic age acceleration; *p* value = *p* value for multiple regression coefficient; R^2 = coefficient of determination for the latent condition count slope; *SE* = standard error.

Table 2. Chronic Condition Slope predicted by Latent Loneliness Mediated Through Different EAA Measures

Predictor	Hannum acceleration $R^2 = 0.035$		Horvath acceleration $R^2 = 0.032$		PhenoAge acceleration $R^2 = 0.042$		GrimAge acceleration $R^2 = 0.040$		DunedinPoAm38 $R^2 = 0.034$		EAA average $R^2 = 0.044$	
	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value	β (*SE*)	*p* Value
Loneliness indirect effect	0.004 (0.002)	.07	0.002 (0.002)	.32	0.008 (0.004)	.04	**0.020 (0.007)**	**.003**	0.008 (0.004)	.03	**0.018 (0.006)**	**.001**
EAA	**0.06 (0.03)**	**.02**	0.04 (0.03)	.14	**0.11 (0.03)**	**<.001**	**0.12 (0.03)**	**.001**	**0.08 (0.03)**	**.005**	**0.13 (0.03)**	**<.001**
Chronological age	**0.18 (0.03)**	**<.001**	**0.18 (0.03)**	**<.001**	**0.18 (0.03)**	**<.001**	**0.17 (0.03)**	**<.001**	**0.17 (0.03)**	**<.001**	**0.17 (0.03)**	**<.001**
Gender (1 = female, 0 = male)	0.01 (0.03)	.81	0.00 (0.03)	.94	0.01 (0.03)	.86	0.01 (0.03)	.71	0.00 (0.03)	.99	0.01 (0.03)	.67
Race (1 = Black, 0 = Other)	0.06 (0.04)	.10	0.05 (0.04)	.15	0.05 (0.04)	.16	0.04 (0.04)	.30	0.05 (0.04)	.18	0.05 (0.04)	.17
Race (1 = White, 0 = Other)	0.03 (0.04)	.37	0.03 (0.04)	.38	0.03 (0.04)	.36	0.03 (0.04)	.42	0.04 (0.04)	.34	0.03 (0.04)	.37
School years	0.04 (0.03)	.18	0.04 (0.03)	.20	0.04 (0.03)	.15	0.05 (0.03)	.11	0.04 (0.03)	.17	0.05 (0.03)	.12
Smoking volume	0.01 (0.03)	.81	0.01 (0.03)	.76	0.01 (0.03)	.89	−0.01 (0.04)	.86	0.00 (0.03)	.91	−0.01 (0.03)	.81
Avg # drinks	0.04 (0.03)	.22	0.04 (0.03)	.24	0.03 (0.03)	.32	0.02 (0.03)	.52	0.03 (0.03)	.34	0.02 (0.03)	.42
Alcohol frequency	−0.02 (0.03)	.59	−0.02 (0.03)	.61	−0.02 (0.03)	.54	−0.01 (0.03)	.71	−0.01 (0.03)	.74	−0.01 (0.03)	.68
BMI	0.05 (0.03)	.12	0.05 (0.03)	.10	0.14 (0.03)	.16	0.05 (0.03)	.17	0.05 (0.03)	.13	0.04 (0.03)	.20

Notes: Results that are statistically significant after accounting for the false discovery rate are printed in bold, given six comparisons and $\alpha = 0.05$. Loneliness was measured in 2010, 2014, and 2018 for half the sample and 2008, 2012, and 2016 for the other half and operationalized as a linear growth curve across three timepoints (2008/10, 2012/14, and 2016/18). Gender was self-reported as a binary variable (1 = female; 0 = male). Given small samples across groups, race was coded with two binary variables. Number of years of education (*School years*) was self-reported. *Smoking volume* is operationalized as number of cigarettes per day (0 for nonsmokers). *Avg # drinks* was self-reported by asking participants about their typical amount of alcohol consumption when they drink. *Alcohol frequency* was self-reported by asking participants, "In the last three months, on average, how many days per week have you had any alcohol to drink?" (0–7). BMI was calculated using participant's self-reported heights and weights. Root mean square error of approximation (RMSEA) ranged from 0.026 to 0.039 across models. β = standardized multiple regression coefficient; BMI = body mass index; EAA = epigenetic age acceleration; *p* value = *p* value for multiple regression coefficient; R^2 = coefficient of determination for the latent condition count slope; *SE* = standard error.

variable, predicted by EAA and the specified covariate set directly and indirectly by the loneliness intercept through EAA for inference on mediation. Bidirectional paths were modeled between each of the latent variables to account for associations between multimorbidity and loneliness, as well as between each construct's latent intercept and slope. See Figure 1 for an example path diagram.

The third set of models examined whether EAA moderated the association between loneliness and multimorbidity change. To do so, a statistical interaction term between the loneliness intercept and EAA was included as a predictor of the condition count slope, along with the main effect of EAA and the specified covariate set. As before, bidirectional paths between each of the latent constructs were modeled. Because the loneliness intercept was a latent rather than observed variable, the interaction term was also latent and modeled using the "xwith" function in Mplus. To do so, we used the default Mplus numerical integration algorithm (an analytic solution is undefinable for an interaction between a latent and observed variable). To decrease collinearity, the loneliness and EAA variables were standardized prior to the derivation of the interaction term. Otherwise, variables were not standardized prior to analyses.

Within each set of models, we accounted for multiple testing across the six EAA measures by applying a 0.05 false discovery rate (FDR) correction (Benjamini & Hochberg, 1995). In all analyses, we used the HRS-derived sample weights to compensate for unequal probabilities of selection across the complex survey design (Fisher & Ryan, 2018). Models were estimated using full information maximum likelihood with robust standard errors (MLR) to account for non-normality. Estimation of each terminated normally. Root mean square errors of approximation ranged from 0.029 to 0.046,

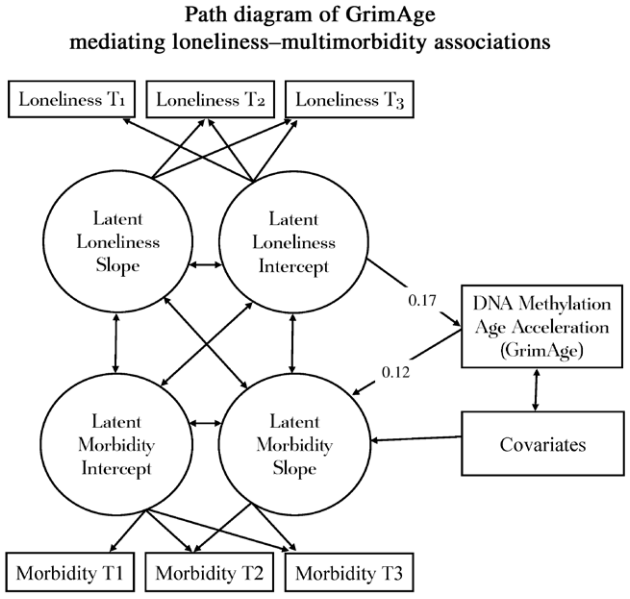

Figure 1. Full model results are displayed in Table 2. Covariates included chronological age, gender, race, education, BMI, smoking, and alcohol use. Some paths are missing from the diagram for simplicity (i.e., residual paths and paths between covariates and latent variables). T1, T2, and T3 refer to chronological sampling timepoints, discussed in Method section. Standardized estimates (β) are displayed for the paths corresponding to the hypothesis that GrimAge acceleration mediates the association between loneliness and increases in chronic condition counts (multimorbidity) over time (indirect effect β = 0.020, p = .003). BMI = body mass index; DNA = deoxyribonucleic acid.

suggesting at least adequate fit to the data in the first and second sets of models. Absolute fit indices were not applicable for the third set of models due to the numerical integration algorithm. Across models, we report standardized regression coefficients and standard errors for all independent variables that were regressed on the focal dependent variable (i.e., EAA in the first set of models and the condition count slope in the second and third sets), as well as that dependent variable's coefficient of determination (R^2).

Results

Associations Between Loneliness and EAA

Each EAA measure had a small, nominally positive association with the latent loneliness intercept, net of demographic and behavioral covariates ($0.03 \le \beta \le 0.07$). The associations were statistically significant after accounting for the FDR with GrimAge (β = 0.07, p = .003) and the EAA average measure (β = 0.06, p = .01). The loneliness slope was not a significant predictor of EAA, though the association was of greatest magnitude for GrimAge as well (β = 0.08, p = .04). Loneliness and demographics accounted for a small to moderate amount of variance in GrimAge (R^2 = 0.17) and EAA average (R^2 = 0.08), but only a marginal amount in the other four EAA measures (range of $R^2 < 0.05$). The addition of behavioral covariates slightly to moderately increased the variance accounted for in GrimAge (R^2 = 0.32), DunedinPoAm38 (R^2 = 0.16), and EAA average (R^2 = 0.15), but not the earlier measures (PhenoAge, Hannum, and Horvath $R^2 < 0.06$). Results for these six models are reported in Table 1, and results for models that do not include behavioral covariates are reported in Supplementary Table 4.

Female gender was significant predictor of negative EAA (i.e., age deceleration) across measures ($-0.32 \le \beta \le -0.07$). Higher level of education was also a significant predictor of negative EAA in later-generation measures ($-0.14 \le \beta \le -0.08$), but not earlier EAA measures ($-0.04 \le \beta \le 0.04$). Associations between race and EAA were inconsistent ($-0.11 \le \beta \le 0.11$). Smoking a greater number of cigarettes per day was a moderate predictor of greater EAA in later measures ($0.27 \le \beta \le 0.39$), but only a modest predictor in earlier EAA measures ($0.03 \le \beta \le 0.06$). Higher BMI was a significant predictor of greater EAA across five of six measures ($0.04 \le \beta \le 0.12$). Finally, greater volume of alcohol consumption significantly predicted greater EAA in later measures ($0.06 \le \beta \le 0.12$) but drinking frequency did not ($-0.05 \le \beta \le 0.02$).

EAA Mediating Loneliness–Multimorbidity Associations

Because loneliness (r = 0.28) and EAA ($0.04 \le r \le 0.19$) both had significant bivariate associations with condition counts (Supplementary Table 3), as well as several individual conditions (Supplementary Table 2), we next considered models that test whether EAA might plausibly mediate loneliness–multimorbidity associations. Net of demographic and behavioral covariates, greater EAA was a predictor of a positive slope of conditions (β = 0.04–0.13; significant for five of six measures). The indirect association between the loneliness intercept and the conditions slope mediated through EAA was nominally positive across measures (β = 0.002–0.020) and statistically significant through GrimAge (β = 0.020, p = .003) and the composite measure (β = 0.018, p = .001).

See Figure 1 for the path diagram depicting GrimAge results. Among the covariates, only older chronological age (β = 0.17–0.18) was a significant predictor of the condition count slope. Results for these six models are reported in Table 2, and results for models that do not include behavioral covariates are reported in Supplementary Table 5. Models accounted for 3%–5% of the variance in the condition count slope.

EAA Moderating Loneliness–Multimorbidity Associations

The final set of models included a multiplicative interaction term between the loneliness intercept and EAA as a predictor of the conditions slope to test for the possibility of DNA methylation as a moderator of loneliness–health associations. The interaction term was not a significant predictor of the conditions slope with any EAA measure across both covariate sets ($-0.04 \leq \beta \leq 0.03$). Demographic covariate model results are reported in the top half of Supplementary Table 6, and models results with behavioral covariates are in the bottom half.

Discussion

Our primary aim was to explore if variability in DNA methylation could contribute to links between loneliness and adverse health outcomes late in life. We first quantified associations between loneliness and epigenetic aging, before relating both variables to changes in multimorbidity. In relating loneliness to multimorbidity, we considered the possibility of both statistical mediation and moderation by EAA. We first conducted analyses using a set of demographic covariates, and then added behavioral predictors to determine how factors like diet and exercise (as reflected by BMI), alcohol consumption, and smoking contributed to the loneliness–health associations.

Baseline loneliness was weakly associated with accelerated epigenetic aging across measures, meeting significance thresholds in GrimAge and a composite after accounting for the FDR. The loneliness slopes were not significantly associated with EAA, though a nominally positive effect of similar magnitude was observed with GrimAge. Freilich et al. (2024) also observed weak associations between loneliness and EAA that were more pronounced in GrimAge and DunedinPACE. Across both studies, health behavior slightly attenuated associations. Similarly, despite using a different longitudinal model, Lynch et al. (2023) observed small associations in HRS between EAA and loneliness, which were most pronounced for GrimAge. Loneliness and EAA were weak to moderate predictors of increases in chronic conditions, consistent with prior work (Freilich et al., 2024; Hajek et al., 2020). Across all analyses, later-generation EAA measures like GrimAge and the Dunedin indices tended to have stronger associations with expected correlates (e.g., male gender, lower education, cigarette smoking, higher BMI), as well as with loneliness and multimorbidity, suggesting they may more effectively capture the epigenetic impacts of psychosocial stressors. Results from both studies further support the finding that lonely individuals face poorer health outcomes, and, on average, experience accelerated epigenetic aging (Beach et al., 2022; Wang et al., 2023).

Much uncertainty remains about whether loneliness leads to health decline mechanistically through impacts on DNA methylation. To test mediation, we modeled indirect effects of loneliness on change in condition counts through EAA; indirect effects were nominally positive across measures, meeting significance thresholds for GrimAge and the composite. Though Freilich et al. (2024) did not observe significant indirect paths, the magnitudes of effects were nominally similar. Detection of a subtle signal in HRS might have been possible because of its relatively older or larger sample or because latent change in loneliness could be modeled. Additionally, Phillips (2020) and Lynch et al. (2023) found that methylation levels at specific CpG sites and EAA, respectively, mediated associations between loneliness and cognitive health with similarly small effect sizes. Extending these Lynch et al. (2023) findings indicates that the explanatory power of EAA for loneliness–health associations in the HRS sample is robust across certain analytic methods and outcome measures.

Loneliness has been theorized to impact health through epigenetic pathways due to its links with hypervigilance in the HPA axis (Eachus & Cunliffe, 2018), as well as with inflammatory and antiviral gene expression (Cole et al., 2007). Cole (2014) summarized this body of research, proposing a pathway by which the perception of psychosocial adversity can theoretically lead to differential transcription factor activity and subsequent inflammatory and immunological responses associated with poorer health. Transcription factors also influence DNA methylation by recruiting DNA methyltransferases onto the genome (Moore et al., 2013), suggesting similar pathways from psychosocial adversity to differential methylation. Altogether, growing evidence suggests that psychosocial adversities may become biologically embedded to have chronic health impacts by affecting gene expression. Recent measures like GrimAge appear to offer a promising index of the health-relevant epigenetic variation. At the same time, however, associations between loneliness and cardiovascular/inflammatory conditions were of similar magnitude as with respiratory, neurological, and musculoskeletal conditions in the current sample (Supplementary Table 2), suggesting other relevant pathways.

Rather than (or in addition to) operating as a mediator, it is plausible that epigenetic variability could affect biological responses to loneliness in a manner that increases individual disease vulnerability. To test for this kind of synergistic effect, we used a statistical interaction between baseline loneliness and EAA as a predictor of multimorbidity. In contrast to Freilich et al. (2024), moderation results were null across measures and no clear pattern emerged. This failure to replicate raises the possibility of a type-I error, but also may reflect the difficulty inherent in detecting subtle interactive effects due to the impact of measurement error across two variables (McClelland & Judd, 1993), especially in the context of modeling complex human traits (Borsboom, 2006).

Loneliness–health associations likely are partially attributable to health-limiting behaviors, demographic confounding, and genetic overlap. We used two additive covariate sets to partially control for the first two possibilities. Indeed, loneliness–health associations were slightly attenuated by the addition of behavioral covariates, though the degree to which overlapping genetic architectures may account for the observed associations is unknown. The impacts of all these effects are likely subtle and perhaps interactive, rather than there being a singular mechanism by which loneliness causes physical decline (Cacioppo et al., 2002; Hawkley & Cacioppo, 2010). More research will be needed to understand the role of epigenetics in the relationship, but these preliminary results are consistent

with a pathway from experiencing loneliness to health decline partially involving changes in DNA methylation.

Limitations

DNA methylation was profiled at only one timepoint, so definitive conclusions about temporal sequencing in its relationships with loneliness and multimorbidity are premature. Loneliness and condition counts were measured across three waves, allowing us to model EAA (2016) as a mediator temporally between baseline loneliness (2008/2010) and change in multimorbidity (2012–2016–2020). However, it is unclear whether the relevant EAA variability emerged in this sequence. Future studies would benefit from repeated measurement, though, even then, other noncausal interpretations of this patterning of results are possible. A further limitation of the current models was the necessity of a measurement wave time metric. Individually varying metrics might allow for flexible modeling of developmental processes, rather than collapsing heterogeneity in age within the waves.

Further, methylation was profiled from circulating blood samples; it is unknown how effects would vary across samples from different tissues as methylation is fundamentally specific to a given cell type. Full information maximum likelihood was used for all models, which assumes data are missing at random. However, loneliness means varied slightly across levels of missingness, possibly biasing estimates. Measurement error is present for all observed variables, many of which are self-reported, also raising the risk of biasing results. Most of the reported conditions, for instance, can be considered largely objective (e.g., heart attack, stroke), but associations between loneliness and the more subjective ailments (e.g., chronic pain, psychiatric problems) could be artificially inflated by evaluative consistency. In addition, condition counts may not capture the health impact of loneliness or perhaps may artifactually overstate the impacts, as it was interpreted as a partially generalized proxy for overall health in older adulthood. Future studies should consider other outcomes both similarly broad and within specific systems to better understand loneliness' impacts on health and well-being across the lifespan.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series B: Psychological Sciences and Social Sciences* online.

Author Notes

1. The study's first aim conceptually replicates Lynch et al.'s (2023) work in HRS with a different longitudinal model. While Lynch et al. (2023) defined loneliness subgroups using growth mixture modeling, we chose to fit linear growth models that do not assume the existence of distinctive latent loneliness classes.

2. Missing data for loneliness was minimal within measurement occasions. Across the six waves, participants filled out at least one loneliness item on 7,636 occasions. Of those 7,636 potential measurements, 11 were excluded (0.14%) because six or more items were missing. Imputation was necessary for five items on four occasions (0.05%), for four items on six occasions (0.08%), for three items on 21 occasions (0.28%), for two items on 42 occasions (0.55%), for one item on 272 occasions (3.56%), and no imputation was necessary on 7,280 occasions (95.34%).

3. At Timepoint 1, mean loneliness scores were 16.5, 16.6, and 17.5 for individuals who provided data across all three waves (no missingness) compared with two (one missing wave) and one wave (two missing waves), respectively (standard deviations = 4.72–4.81). Mean differences were small to medium ($t = 0.37–2.11$, $p = .04–.71$, $d = 0.02–0.22$). These results suggest that data may not be missing completely at random; individuals with more missing data, are, on average, slightly lonelier, possibly biasing results that use full information maximum likelihood estimation.

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Conflict of Interest

None.

Data Availability

Raw data are available to the public through https://hrs.isr.umich.edu/. Processed data and Mplus output for all models are provided at https://osf.io/yj759/?view_only=01226fb92fb044aaad7f3be7d49b671c. This study's design and its analysis were not preregistered. The larger Health and Retirement Study protocol was reviewed and approved by the University of Michigan IRB; the current study was exempt from an IRB review because we used publicly available, deidentifiable data.

References

Abdellaoui, A., Sanchez-Roige, S., Sealock, J., Treur, J. L., Dennis, J., Fontanillas, P., Elson, S., Nivard, M. G., Ip, H. F., van der Zee, M., Baselmans, B. M. L., Hottenga, J. J., Willemsen, G., Mosing, M., Lu, Y., Pedersen, N. L., Denys, D., Amin, N., & Boomsma, D. I. (2019). Phenome-wide investigation of health outcomes associated with genetic predisposition to loneliness. *Human Molecular Genetics*, 28(22), 3853–3865. https://doi.org/10.1093/hmg/ddz219

Allen, J. P., Danoff, J. S., Costello, M. A., Loeb, E. L., Davis, A. A., Hunt, G. L., Gregory, S. G., Giamberardino, S. N., & Connelly, J. J. (2022). Adolescent peer struggles predict accelerated epigenetic aging in midlife. *Development and Psychopathology*, 35, 912–925. https://doi.org/10.1017/s0954579422000153

Beach, S. R. H., Klopack, E. T., Carter, S. E., Philibert, R. A., Simons, R. L., Gibbons, F. X., Ong, M. L., Gerrard, M., & Lei, M.-K. (2022). Do loneliness and per capita income combine to increase the pace of biological aging for Black Adults across late middle age? *International Journal of Environmental Research and Public Health*, 19(20), 13421. https://doi.org/10.3390/ijerph192013421

Belsky, D. W., Caspi, A., Arseneault, L., Baccarelli, A., Corcoran, D. L., Gao, X., Hannon, E., Harrington, H. L., Rasmussen, L. J., Houts, R., Huffman, K., Kraus, W. E., Kwon, D., Mill, J., Pieper, C. F., Prinz, J. A., Poulton, R., Schwartz, J., Sugden, K., … Moffitt, T. E. (2020). Quantification of the pace of biological aging in humans

through a blood test, the DunedinPoAm DNA methylation algorithm. *eLife*, 9, e54870. https://doi.org/10.7554/eLife.54870

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B: Statistical Methodology*, 57(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Borsboom, D. (2006). The attack of the psychometricians. *Psychometrika*, 71(3), 425–440. https://doi.org/10.1007/s11336-006-1447-6

Cacioppo, J. T., Hawkley, L. C., Crawford, L. E., Ernst, J. M., Burleson, M. H., Kowalewski, R. B., Malarkey, W. B., Van Cauter, E., & Berntson, G. G. (2002). Loneliness and health: Potential mechanisms. *Psychosomatic Medicine*, 64(3), 407–17. https://doi.org/10.1097/00006842-200205000-00005

Capitanio, J. P., Cacioppo, S., & Cole, S. W. (2019). Loneliness in monkeys: Neuroimmune mechanisms. *Current Opinion in Behavioral Sciences*, 28, 51–57. https://doi.org/10.1016/j.cobeha.2019.01.013

Cole, S. W. (2014). Human social genomics. *PLoS Genetics*, 10(8), e1004601. https://doi.org/10.1371/journal.pgen.1004601

Cole, S. W., Hawkley, L. C., Arevalo, J. M., Sung, C. Y., Rose, R. M., & Cacioppo, J. T. (2007). Social regulation of gene expression in human leukocytes. *Genome Biology*, 8(9), R189. https://doi.org/10.1186/gb-2007-8-9-r189

Crimmins, E., Faul, J., Thyagarajan, B., & Weir, D. (2017). *Venous blood collection and assay protocol in the 2016 Health and Retirement Study*. Survey Research Center, Institute for Social Research, University of Michigan.

Doane, L. D., Mineka, S., Zinbarg, R. E., Craske, M., Griffith, J. W., & Adam, E. K. (2013). Are flatter diurnal cortisol rhythms associated with major depression and anxiety disorders in late adolescence? The role of life stress and daily negative emotion. *Development and Psychopathology*, 25(3), 629–642. https://doi.org/10.1017/s0954579413000060

Duman, E. A., & Canli, T. (2015). Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. *Biology of Mood and Anxiety Disorders*, 5, 2. https://doi.org/10.1186/s13587-015-0017-x

Eachus, H., & Cunliffe, V. T. (2018). Chapter 12: Biological embedding of psychosocial stress over the life course. In A. Moskalev & A. M. Vaiserman (Eds.), *Epigenetics of aging and longevity* (Vol. 4, pp. 251–270). Academic Press. https://doi.org/10.1016/B978-0-12-811060-7.00012-7

Faul, J. D., Kim, J. K., Levine, M. E., Thyagarajan, B., Weir, D. R., & Crimmins, E. M. (2023). Epigenetic-based age acceleration in a representative sample of older Americans: associations with aging-related morbidity and mortality. *Proceedings of the National Academy of Sciences of the United States of America*, 120(9), e2215840120. https://doi.org/10.1073/pnas.2215840120

Fisher, G. G., & Ryan, L. H. (2018). Overview of the health and retirement study and introduction to the special issue. *Work, Aging and Retirement*, 4(1), 1–9. https://doi.org/10.1093/workar/wax032

Freilich, C. D. (2023). How does loneliness "get under the skin" to become biologically embedded? *Biodemography and Social Biology*, 68, 115–148. https://doi.org/10.1080/19485565.2023.2260742

Freilich, C. D., Markon, K. E., Cole, S. W., & Krueger, R. F. (2024). Loneliness, epigenetic age acceleration, and chronic health conditions. *Psychology and Aging*, 39(4), 337–349. https://doi.org/10.1037/pag0000822

Hajek, A., Kretzler, B., & König, H.-H. (2020). Multimorbidity, loneliness, and social isolation. A systematic review. *International Journal of Environmental Research and Public Health*, 17(22), 8688. https://doi.org/10.3390/ijerph17228688

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.-B., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., & Zhang, K. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular Cell*, 49(2), 359–367. https://doi.org/10.1016/j.molcel.2012.10.016

Hawkley, L. C., & Cacioppo, J. T. (2010). Loneliness matters: A theoretical and empirical review of consequences and mechanisms. *Annals of Behavioral Medicine: A Publication of the Society of Behavioral Medicine*, 40(2), 218–227. https://doi.org/10.1007/s12160-010-9210-8

Health and Retirement Study, Venous Blood Study restricted dataset and Survey public use dataset. (2024). Produced and distributed by the University of Michigan with funding from the National Institute on Aging (grant number NIA U01AG009740).

Hillmann, A. R., Dhingra, R., & Reed, R. G. (2023). Positive social factors prospectively predict younger epigenetic age: Findings from the Health and Retirement Study. *Psychoneuroendocrinology*, 148, 105988. https://doi.org/10.1016/j.psyneuen.2022.105988

Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., & Stephenson, D. (2015). Loneliness and social isolation as risk factors for mortality: A meta-analytic review. *Perspectives on Psychological Science*, 10(2), 227–237. https://doi.org/10.1177/1745691614568352

Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biology*, 14(10), Article 10. https://doi.org/10.1186/gb-2013-14-10-r115

Juster, F. T., & Suzman, R. (1995). An overview of the health and retirement study. *The Journal of Human Resources*, 30, S7–S56. https://doi.org/10.2307/146277

Krieger, N., Chen, J. T., Testa, C., Diez Roux, A., Tilling, K., Watkins, S., Simpkin, A. J., Suderman, M., Davey Smith, G., De Vivo, I., Waterman, P. D., & Relton, C. (2023). Use of correct and incorrect methods of accounting for age in studies of epigenetic accelerated aging: Implications and recommendations for best practices. *American Journal of Epidemiology*, 192(5), 800–811. https://doi.org/10.1093/aje/kwad025

Lai, J. C. L., Lee, D. Y. H., Leung, M. O. Y., & Lam, Y. W. (2019). Daily hassles, loneliness, and diurnal salivary cortisol in emerging adults. *Hormones and Behavior*, 115, 104558. https://doi.org/10.1016/j.yhbeh.2019.07.006

Levine, M. E., Lu, A. T., Quach, A., Chen, B. H., Assimes, T. L., Bandinelli, S., Hou, L., Baccarelli, A. A., Stewart, J. D., Li, Y., Whitsel, E. A., Wilson, J. G., Reiner, A. P., Aviv, A., Lohman, K., Liu, Y., Ferrucci, L., & Horvath, S. (2018). An epigenetic biomarker of aging for lifespan and healthspan. *Aging*, 10(4), 573–591. https://doi.org/10.18632/aging.101414

Li, H., & Xia, N. (2020). The role of oxidative stress in cardiovascular disease caused by social isolation and loneliness. *Redox Biology*, 37, 101585. https://doi.org/10.1016/j.redox.2020.101585

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. https://doi.org/10.18632/aging.101684

Luo, Y., Hawkley, L. C., Waite, L. J., & Cacioppo, J. T. (2012). Loneliness, health, and mortality in old age: A national longitudinal study. *Social Science & Medicine*, 74(6), 907–914. https://doi.org/10.1016/j.socscimed.2011.11.028

Luo, Y., & Waite, L. J. (2014). Loneliness and mortality among older adults in China. *The Journals of Gerontology, Series B: Psychological Sciences and Social Sciences*, 69(4), 633–645. https://doi.org/10.1093/geronb/gbu007

Lynch, M., Em Arpawong, T., & Beam, C. R. (2023). Associations between longitudinal loneliness, DNA methylation age acceleration, and cognitive functioning. *The Journals of Gerontology, Series B: Psychological Sciences and Social Sciences*, 78(12), 2045–2059. https://doi.org/10.1093/geronb/gbad128

Marengoni, A., Angleman, S., Melis, R., Mangialasche, F., Karp, A., Garmen, A., Meinow, B., & Fratiglioni, L. (2011). Aging with multimorbidity: A systematic review of the literature. *Ageing Research Reviews*, 10(4), 430–439. https://doi.org/10.1016/j.arr.2011.03.003

McClelland, G. H., & Judd, C. M. (1993). Statistical difficulties of detecting interactions and moderator effects. *Psychological Bulletin*, 114(2), 376–390. https://doi.org/10.1037/0033-2909.114.2.376

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology*, 38(1), 23–38. https://doi.org/10.1038/npp.2012.112

Muthén, L. K., & Muthén, B. O. (2024). *Mplus user's guide*. 8th ed. Muthén & Muthén. https://www.statmodel.com/html_ug.shtml

Nakamura, J. S., Kwok, C., Huang, A., Strecher, V. J., Kim, E. S., & Cole, S. W. (2023). Reduced epigenetic age in older adults who volunteer. *Psychoneuroendocrinology*, 148, 106000. https://doi.org/10.1016/j.psyneuen.2022.106000

Oblak, L., van der Zaag, J., Higgins-Chen, A. T., Levine, M. E., & Boks, M. P. (2021). A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing Research Reviews*, 69, 101348. https://doi.org/10.1016/j.arr.2021.101348

Office of the Surgeon General (OSG). (2023). *Our epidemic of loneliness and isolation: The U.S. Surgeon General's advisory on the healing effects of social connection and community*. US Department of Health and Human Services. http://www.ncbi.nlm.nih.gov/books/NBK595227/

O'Sullivan, R., Leavey, G., & Lawlor, B. (2022). We need a public health approach to loneliness. *BMJ*, 376, o280. https://doi.org/10.1136/bmj.o280

Patterson, A. C., & Veenstra, G. (2010). Loneliness and risk of mortality: A longitudinal investigation in Alameda County, California. *Social Science & Medicine (1982)*, 71(1), 181–186. https://doi.org/10.1016/j.socscimed.2010.03.024

Phillips, D. M. (2020). *Longitudinal loneliness and cognitive aging in mid and late life: patterns of associations and epigenetic pathways* [*PhD*, University of California, Riverside]. https://www.proquest.com/docview/2406985532/abstract/3B49C18A9F-744C04PQ/1

Russell, D. W. (1996). UCLA Loneliness Scale (version 3): Reliability, validity, and factor structure. *Journal of Personality Assessment*, 66(1), 20–40. https://doi.org/10.1207/s15327752jpa6601_2

Unternaehrer, E., Luers, P., Mill, J., Dempster, E., Meyer, A. H., Staehli, S., Lieb, R., Hellhammer, D. H., & Meinlschmidt, G. (2012). Dynamic changes in DNA methylation of stress-associated genes (OXTR, BDNF) after acute psychosocial stress. *Translational Psychiatry*, 2(8), e150. https://doi.org/10.1038/tp.2012.77

Wang, F., Gao, Y., Han, Z., Yu, Y., Long, Z., Jiang, X., Wu, Y., Pei, B., Cao, Y., Ye, J., Wang, M., & Zhao, Y. (2023). A systematic review and meta-analysis of 90 cohort studies of social isolation, loneliness and mortality. *Nature Human Behaviour*, 7(8), 1307–1319. https://doi.org/10.1038/s41562-023-01617-6