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# Loneliness, Epigenetic Age Acceleration, and Chronic Health Conditions

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Having associations with a range of adverse physical health outcomes including mortality, loneliness is increasingly recognized as a pressing public health concern, but the mechanisms studied to date do not yet explain all loneliness-related health risk. We sought to evaluate whether epigenetic influences on DNA methylation could help explain the relationship between loneliness and health. To do so, we first estimated associations between loneliness and epigenetic age acceleration (EAA) in a subsample of participants in the study of midlife in the United States ($n$ = 1,310), before testing whether EAA mediated and/or moderated the association between loneliness and the onset of chronic health conditions in older adulthood ($n$ = 445 completing longitudinal follow-ups). Greater loneliness was weakly associated with greater EAA in the Horvath, DunedinPACE, and GrimAge measures after accounting for demographic ($0.08 \leq \beta \leq 0.11$) and behavioral ($0.06 \leq \beta \leq 0.08$) covariates. Loneliness also predicted increases in chronic condition counts and these effects were more pronounced for individuals with higher DunedinPACE EAA values (interaction term $\beta = 0.09$, $p = .009$), suggesting possible synergistic impacts. EAA measures appear to be promising in helping to understand individual variations in the health impacts of loneliness, but the specific mechanisms involved require further research.

***Public Significance Statement***

Lonely individuals face poorer health outcomes than nonlonely individuals. These data support the notion that loneliness is associated with accelerated epigenetic aging which may amplify the impact of loneliness on physical health in older adulthood.

*Keywords:* loneliness, epigenetic age acceleration, epigenetic clock, biological embedding

*Supplemental materials:* https://doi.org/10.1037/pag0000822.supp



## Public Health Relevance of Loneliness and Potential Mechanisms

Loneliness has been defined as the distress arising from the perception that one's social needs are not being met by their relationships (Hawkley & Cacioppo, 2010). The feeling or emotional experience of loneliness can be reliably indexed with self-report scales and is distinct from objective social isolation (i.e., frequency of social contact; Russell, 1996). Self-reports of loneliness have been associated with a range of physical health measures, including ones across cardiovascular (Hodgson et al., 2020; Valtorta et al., 2016), inflammatory (Smith et al., 2020; Vingeliene et al., 2019), metabolic (Shiovitz-Ezra & Parag, 2019; Whisman, 2010), and other self-reported (e.g., subjective health, frailty; Gale et al., 2018; Nummela et al., 2011) domains, as well as with early mortality (Holt-Lunstad et al., 2015). In fact, in a recent meta-analysis, loneliness was

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Raw data are available to the public through the Colectica portal at https://midus.colectica.org/. Processed data and Mplus output for all models are provided at https://osf.io/znqmy/. This study's design and its analysis were not preregistered. The ideas and data appearing in the article were presented in a preliminary form during a talk at the Behavior Genetics Association Conference in June 2023.

Colin D. Freilich played a lead role in formal analysis, investigation, methodology, writing–original draft, and writing–review and editing and an equal role in conceptualization. Kristian E. Markon played a supporting role in methodology and validation and an equal role in writing–review and editing. Steve W. Cole played a lead role in data curation, a supporting role in methodology, and an equal role in conceptualization, funding acquisition, and writing–review and editing. Robert F. Krueger played a lead role in project administration, software, supervision and validation, a supporting role in data curation, and an equal role in conceptualization, funding acquisition, and writing–review and editing.

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significantly associated with increased risk for all-cause mortality (pooled effect size: 1.14; 95% confidence interval [1.09, 1.20]) and cancer mortality (1.09 [1.01, 1.17]) and nonsignificantly with cardiovascular disease mortality (1.14 [0.97, 1.35]; Wang et al., 2023).

Loneliness has also been linked with multimorbidity, or the presence of two or more chronic physical health conditions (often operationalized as a count of conditions; Hajek et al., 2020), a particularly important and useful construct in the study of aging given its high prevalence and links with quality of life, disability, and mortality (Marengoni et al., 2011; Salive, 2013). As a physical health outcome, multimorbidity is also useful in studying loneliness, again given its prevalence and salience to overall health, and, additionally, given its generalized nature, likely enabling it to capture some of loneliness's impacts across multiple systems (e.g., inflammatory, cardiovascular, metabolic). Given its robust links with declining health, many, including the U.S. Surgeon General and the National Academies of Sciences, consider loneliness a public health epidemic especially in older adulthood (National Academies Press, 2020; O'Sullivan et al., 2022; U.S. Department of Health & Human Services, 2023).

Multiple mechanisms have been proposed to contribute to loneliness-related health risk. Broadly, psychosocial experiences like loneliness may cause physiological signals that directly result in downstream health effects. Loneliness may also have indirect influences on health via its impact on health-related behaviors (e.g., physical activity and smoking; Luo & Waite, 2014; Patterson & Veenstra, 2010). Some portion of the associations between loneliness and health likely also result from common upstream determinants such as overlapping genetic influences (Abdellaoui et al., 2019) and reverse causality (i.e., poor health influencing social functioning; Holt-Lunstad et al., 2015). However, common determinants, health behaviors, and demographic factors cannot account for the entire loneliness-mortality link, consistent with "loneliness alter(ing) physiology at a more fundamental level" (Freilich, 2023b; Luo et al., 2012, p. 912).

## Loneliness and Gene Expression: Prior Evidence

In an evolutionary conception of loneliness, Hawkley and Cacioppo's (2010) posit that feeling socially connected is tantamount to feeling safe, and, therefore, experiencing loneliness sets off hypervigilant physiological responses to (social) environmental threats, directly impacting health. Lonely individuals are, indeed, more susceptible to perceiving common events as stressful (Cacioppo, 1994), and this may lead to activation of physiological stress response systems such as the sympathetic nervous system (SNS) and hypothalamic–pituitary–adrenocortical axis (Eachus & Cunliffe, 2018). Though effects are somewhat inconsistent across studies, loneliness has been linked to differential cortisol levels (Kiecolt-Glaser et al., 1984; Lai et al., 2018, 2019; Pressman et al., 2005; Steptoe et al., 2004) and diurnal variation (Doane & Adam, 2010; Doane et al., 2013), as well as elevated levels of SNS neuroeffector molecules such as norepinephrine (Capitanio et al., 2019; Cole et al., 2015). SNS and hypothalamic–pituitary–adrenocortical axis dysregulation can in turn impact physiological function and disease development by altering gene transcriptional processes involved in proinflammatory signaling pathways (Rhen & Cidlowski, 2005). Several studies have linked loneliness to elevated activity of proinflammatory transcription factors and reduced activity of antiviral transcription factors (Cole et al., 2007, 2010; Heidt et al., 2014; Powell et al., 2013), suggesting that loneliness may impact health through gene regulatory (epigenetic) pathways.

## Loneliness, Methylation, and Multimorbidity

One way gene transcription is regulated is through the binding of methyl group molecules to DNA. When a methyl group binds to a particular gene, it can prevent that gene's transcription into RNA, in effect regulating the expression of that gene as functional protein products. Methylation levels can vary (0%−100%) in regions of the genome called CpG islands (named for their high frequency of Cytosine followed by Guanine nucleotides, linked by Phosphate), and some CpG islands show progressive decreases in DNA methylation with aging (Heyn et al., 2012). This observation led to the development of "epigenetic clocks" that assess biological age as distinct from chronological age. "First-generation" epigenetic clocks were formed by linear combinations of CpG methylation intensities to optimally predict chronological age (e.g., the Horvath, 2013 and Hannum et al., 2013 epigenetic clocks). To the extent that epigenetic clock values exceeded an individual's chronological age, the individual is said to show "epigenetic age acceleration" (EAA), and EAA has come to be widely studied in the context of aging (Oblak et al., 2021).

To better predict future disease or mortality, "second-generation" EAA measures were developed to optimally predict phenotypic characteristics of aging such as disease incidence, longevity, or disability. For instance, a "phenotypic age" clock (PhenoAge) was developed by predicting various clinical health markers (e.g., the C-reactive protein indicator of inflammation, metabolic glucose levels, white blood cell counts) in addition to chronological age, resulting in an epigenetic clock that theoretically was a stronger proxy for biological or functional age (Levine et al., 2018). Similarly, GrimAge was trained on age and various clinical makers, with a focus on plasma proteins that have previously been associated with mortality or morbidity (Lu et al., 2019). The DunedinPACE measure was developed to predict within-individual decline in indicators of organ-system integrity (Belsky et al., 2022). Unlike the other "clocks," DunedinPACE is calculated as a ratio of an individual's rate or pace of aging, that is, the number of "biological years" they are currently aging per each chronological year ($M$ = 1.00). EAA is moderately heritable (Levine et al., 2015), and second-generation EAA measures predict many adverse physical health outcomes, including all-cause mortality, net of traditional risk factors (Chen et al., 2016).

Research has linked loneliness to elevations in the DunedinPACE measure of EAA (Beach et al., 2022). Galkin et al. (2022) linked a different measure of accelerated biological aging to other negative psychological factors (e.g., "rarely feels happy") but found no association with a binary loneliness item. Reduced EAA has also been linked to other social and relational variables, including social contact, social support, low social strain, and relationship status (Hillmann et al., 2023; Rentscher et al., 2023), attachment styles (Allen et al., 2022), and volunteering status (Nakamura et al., 2023).

EAA has also been linked to several aging and socially patterned health outcomes. Phillips (2020) found that methylation at specific CpG sites might plausibly mediate part of the nonsignificant association between loneliness and longitudinal declines in

processing speed, while Lynch et al. (2023) report data consistent with an indirect effect of certain trajectories of loneliness on future cognitive ability operating through EAA (GrimAge). In both studies, indirect effects were modest, especially when accounting for behavioral covariates which often explain a substantial portion of the association of psychosocial risk factors with EAA. The pathways linking loneliness, EAA, and other physical health outcomes like multimorbidity have not been studied. Like the evidence on cognitive health, methylation may mediate loneliness-disease associations. It is also plausible that methylation might affect biological responses to environmental risk factors and thereby alter individual vulnerability to loneliness-disease associations, consistent with moderation.

## The Present Study

Given paucity of evidence on the role of EAA in the association between loneliness and generalized health outcomes like multimorbidity in older adulthood, we sought to (a) quantify associations between loneliness and several different measures of EAA; (b) quantify associations between EAA and change in chronic health conditions; (c) test whether EAA might plausibly mediate relationships between loneliness and chronic health conditions, and (d) determine whether EAA might potentially moderate the relationship between loneliness and chronic health conditions.

## Method

### Transparency and Openness

The sample size was determined by selecting all participants from the National Survey of Midlife Development in the United States (MIDUS; Brim et al., 2019) with EAA data and, for later models, the subset of those who completed a longitudinal follow-up survey, without any exclusions or manipulations. Raw data are publicly available on the Colectica portal (https://midus.colectica.org/) through the interuniversity Consortium for Political and Social Research (Brim et al., 1995–1996). This study's design and its analysis were not preregistered. Processed data and MPlus syntax and output are available at https://osf.io/znqmy/ (Freilich, 2023a). The larger MIDUS study protocol was reviewed and approved by the Education and Social/Behavioral Sciences and the Health Sciences Institutional Review Boards at the University of Wisconsin–Madison; the present study was exempt from an Institutional Review Board review because we used publicly available, deidentifiable data.

### Participants

The sample includes adults who participated in MIDUS (Brim et al., 2019). MIDUS investigates associations between sociodemographic, psychosocial, and biological variables and later life morbidity and mortality. MIDUS contains multiple waves of data collection across several projects, including the Biomarker Project which involved the assessment of a variety of biological indicators of physiology and health. Within the Biomarker Project and across the Core MIDUS ($n = 511$) and MIDUS Refresher ($n = 799$) cohorts, DNA methylation profiling was conducted on a total of $n = 1{,}310$ participants. In addition, MIDUS survey data were used to measure chronic health conditions across two waves for the Core sample and one wave for the Refresher sample. Taken together, the data stem from three timepoints, referred to as Timepoint 1 survey (2004–2005 for Core, 2011 for Refresher), Timepoint 2 biomarker (2004–2009 for Core, 2012–2016 for Refresher), and Timepoint 3 survey (2013–2017 for Core, not yet conducted for Refresher).

The EAA variables and all covariates (e.g., self-reported level of education and race, smoking and drinking behavior, body mass index [BMI]) stem from Timepoint 2 biomarker. Chronic health condition counts were self-reported at Timepoints 1 and 3 (Survey), and it is the only focal variable available across two timepoints, though for only the Core sample, as the Refresher sample has not yet completed a follow-up survey. Loneliness was measured using indicators from Timepoint 1. The sample at Timepoint 2 biomarker had an average age of 54.0 ($n = 1{,}310$, range 26–86, $SD = 12.6$) and was 55.4% female. Most participants identified as White (69.1%) or Black/African American (22.4%). There was considerable diversity in terms of educational attainment (42.9% without college degree, 35.7% with an undergraduate degree, and 21.2% with a graduate degree). Timepoint 1 was, on average, 2.64 years before Timepoint 2 ($M_{age} = 51.34$, $n = 1{,}310$), while Timepoint 3 was, on average, 7.14 years after Timepoint 2 ($M_{age} = 61.14$, $n = 446$). Descriptive statistics for all observed variables are reported in Supplemental Table S1.

### Measures

#### *Epigenetic Age and EAA*

Fasting blood draws from the Biomarker Project (Timepoint 2) were collected for the Core MIDUS cohort from 2004 to 2009 and for the MIDUS Refresher cohort from 2012 to 2016. Whole blood samples were collected using a BD Vacutainer Tube with EDTA anticoagulant, frozen for storage, and subject to DNA extraction. In 2019, DNA methylation profiling was conducted on the whole blood DNA samples from both the Core and Refresher cohorts. After DNA was tested for suitable yield and integrity, it was subjected to genomewide methylation profiling using Illumina Methylation EPIC microarrays. The resulting "β values" (estimated % methylation at each assayed CpG site) were normalized to control for technical sources of variance, registered onto the list of CpG sites assayed on the Illumina Methylation 450K microarray, and screened using standard quality control metrics. Then, in 2022, the data on methylation profiles were scored using previously published algorithms to compute several measures of "epigenetic age," including the "first-generation" Hannum clock (Hannum et al., 2013) and Horvath clock (Horvath, 2013), the "second-generation" PhenoAge (Levine et al., 2018) and GrimAge (Lu et al., 2019) clocks, and the DunedinPACE measure of EAA (Belsky et al., 2022). For more information on data collection and the derivation of epigenetic age variables in MIDUS, reference the data documentation on the MIDUS Colectica Portal (https://midus.colectica.org/).

While the first four algorithms produce estimates of epigenetic age in years, DunedinPACE is a measure the relative pace of recent aging as a multiplicative factor (i.e., a measure of age acceleration). As a result, the other four clocks correlated strongly ($r \geq 0.89$) with chronological age, while DunedinPACE had a small correlation ($r = 0.18$). Similarly, the four clocks correlated strongly with one another ($r \geq 0.85$) and moderately with DunedinPACE ($0.21 \leq r \leq 0.46$). Descriptive statistics on each of the epigenetic age measures are

reported in Supplemental Table S1. To calculate EAA, the effect of chronological age was regressed out of the four clocks (i.e., epigenetic age net of chronological age), as well as the effect of the individual 96-well plate that was used to store samples for the assay. Well plate was considered a technical covariate treated as a factor, regressed out of each of the five measures. Correlations among these five different measures of EAA (i.e., residuals from regressions of the four clocks on chronological age and well plate and from DunedinPACE on just well plate) are provided in Table 1. In addition, to capture shared variance among the five measures, we included an "EAA average" variable in analyses by taking the arithmetic mean of the five variables.

### *Loneliness*

Loneliness was measured at Timepoint 1 using three items. Participants were asked to indicate "During the past 30 days, how much of the time did you feel [blank]." There were three items: "lonely," "close to others," and "like you belong." Items were rated on a 5-point scale (1 = *none of the time*, 2 = *a little of the time*, 3 = *some of the time*, 4 = *most of the time*, 5 = *all of the time*). A sum score was calculated, reverse coding items when necessary, so that higher scores reflected greater loneliness. Though not a formal loneliness scale, these items resemble those of the often-used University of California Los Angeles Loneliness Scale. For instance, the University of California Los Angeles scale asks participants how often they feel as though they are "completely alone," "no longer close to anyone," and as though "People are around me but not with me" (Russell, 1996). Previously, these items have similarly been used to index loneliness in MIDUS. For instance, the single self-report "lonely" item has been used (Nersesian et al., 2018), as has an identical sum score (Freilich et al., 2023). The three items were strongly correlated ($0.48 < r < 0.77$, $p < .001$).

By asking participants about their experiences over the past 30 days, this scale primarily captures transient negative emotional experiences. Traditional loneliness scales have a relatively high rank-order stability over time, similar to the "traitlike" nature of personality, but tend to ask about general, rather than time-bound, experiences (Mund et al., 2020). Indeed, with longitudinal data, chronic and transient loneliness have been studied concurrently (i.e., contrasting individuals who report loneliness across timepoints with those reporting loneliness across one or a subset of timepoints), with chronic loneliness tending to have a similar but larger association with poorer health than transient loneliness (Martín-María et al., 2020; Zhong et al., 2016). In the broader MIDUS study, the correlation between the three-item sum score used in the present study from Wave 2 to Wave 3 (~9 year interval) is $r(2594) = 0.55$, $p < .001$, similar to a meta-analytic estimate of 5-year stability at midlife (age within 40–60) using traditional loneliness scales ($\rho = 0.63$; Mund et al., 2020). This suggests that, despite being phrased in a time-bound manner, the current scale has a similar "traitlike" stability at midlife into older adulthood and is likely to capture much of the impact of chronic loneliness as would a traditional scale.

### *Number of Chronic Health Conditions (Multimorbidity)*

At Timepoints 1 and 3, participants were asked, "In the past 12 months, have you experienced or been treated for any of the following (check all that apply)" and given a list of 30 separate chronic health conditions (e.g., tuberculosis, joint/bone diseases, varicose veins, migraine headaches, neurological disorder, stroke, swallowing problems). The number of items they selected was used as an index of chronic condition count (i.e., the number of conditions endorsed, unweighted). Multimorbidity, or the presence of two or more chronic medical conditions, is frequently studied in the context of aging as generalized index of health that is common in older adults and has broad correlates (e.g., disability, mortality; Marengoni et al., 2011; Salive, 2013). As expected, we observed an increase in chronic condition count from Timepoint 1 ($M_{conditions} = 2.65$, $M_{age} = 51.3$) to Timepoint 3 ($M_{conditions} = 3.43$, $M_{age} = 61.1$).

## Statistical Analysis

Three sets of models were run across six different EAA variables and two different covariate sets (36 models in total). The first set of multiple linear regression models quantified associations between loneliness (Timepoint 1) and the EAA measures (Timepoint 2). The specified EAA variable ($n = 1{,}310$) was regressed on loneliness and the specified set of covariates. The second set of models tested whether EAA mediated the association between loneliness and change in chronic condition count. To do so, condition count at Timepoint 3 ($n = 445$) was regressed on condition count at

**Table 1**
*Correlations Among Epigenetic Age Acceleration, Loneliness, Health Behaviors, and Chronic Condition Count*

| Variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Hannum acceleration | — | .54* | .50* | .20* | .20* | .03 | .05 | .05 | .03 | .04 | −.04 |
| 2. Horvath acceleration | .54* | — | .47* | .15* | .13* | .08* | .05 | −.01 | .02 | .04 | .00 |
| 3. PhenoAge acceleration | .50* | .47* | — | .41* | .44* | .04 | .16* | .04 | .07* | .15* | .09* |
| 4. GrimAge acceleration | .20* | .15* | .41* | — | .68* | .15* | .51* | .11* | .24* | .10* | .18* |
| 5. DunedinPACE | .20* | .13* | .44* | .68* | — | .14* | .29* | −.08* | .14* | .36* | .20* |
| 6. Loneliness | .03 | .08* | .04 | .15* | .14* | — | .05 | −.03 | .10* | .11* | .33* |
| 7. Smoke pack years | .05 | .05 | .16* | .51* | .29* | .05 | — | .07 | .07* | .04 | .15* |
| 8. Alcohol frequency | .05 | −.01 | .04 | .11* | −.08* | −.03 | .07 | — | .50* | −.18* | −.10* |
| 9. Average no. of drinks | .03 | .02 | .07* | .24* | .14* | .10* | .07* | .50* | — | −.03 | −.03 |
| 10. No. of chronic conditions | .04 | .04 | .15* | .10* | .36* | .11* | .04 | −.18* | −.03 | — | .20* |
| 11. BMI | −.04 | .00 | .09* | .18* | .20* | .33* | .15* | −.10* | −.03 | .20* | — |

*Note.* $n > 1{,}265$ pairwise. Chronic conditions and loneliness were measured at Timepoint 1, while all other variables were measured at Timepoint 2. BMI = body mass index.
* Significant at $p < .01$.

Timepoint 1 (i.e., residual change), as well as the specified EAA variable, loneliness, and the specified set of covariates. In addition, a path was modeled from loneliness to condition count through EAA, allowing for interpretation of both direct and indirect (mediated by EAA) associations. The third set of models examined whether EAA moderated the association between loneliness and change in condition count. To do so, a statistical interaction term between loneliness and EAA was included as a predictor of condition count, along with the main effects of each, previous condition count, and covariates. In deriving the interaction term, the loneliness and EAA variables were standardized to decrease collinearity. Otherwise, variables were not standardized prior to analyses. To account for false discovery and multiple comparisons, we set a significance threshold of $p < .001$ and considered $p < .01$ marginally significant.

Finally, we ran supplementary analyses to explore other factors that may mediate the association between loneliness and health. Given the evidence that health behaviors partially mediate links between loneliness and mortality (Luo & Waite, 2014; Patterson & Veenstra, 2010), we first evaluated smoking behavior, drinking behavior, and BMI as potential mediators of the association between loneliness and residual change in condition count. Next, given robust links between loneliness and personality traits (Buecker et al., 2020), we evaluated neuroticism and extraversion as potential mediators. Finally, given the overlap between loneliness and relational factors, we evaluated self-reported social contact or social isolation and self-reported strain in close relationships as potential mediators. See MIDUS documentation for more information on the measurement of these additional variables (Brim et al., 2019). To do so, we fit multiple linear regressions with loneliness and demographic covariates predicting residual change in chronic condition count and interpreted the indirect paths through the given potential mediator. All models were fit in Mplus Version 8.10 (Muthén & Muthén, 2023) using full information maximum likelihood with robust standard errors. Estimation of all models terminated normally.

## Covariates

All models included demographic factors as covariates. The included demographic variables were self-reported sex, chronological age, race, and level of education. Note that the first set of models (predicting EAA) only included chronological age when DunedinPACE was the outcome because the other EAA variables were calculated by regressing epigenetic age on chronological age. Chronological age was included in each of the second and third sets of models predicting condition counts. Given that most participants identified as White/Caucasian ($n = 905$, 69.1%) or Black/African American ($n = 294$, 22.4%), race was coded as a binary variable (1 = racially minoritized, 0 = White/Caucasian). Education was self-reported on an ordinal scale ranging from 1 to 12; for example, 1 = *no school/some grade school* (1–6); 8 = *graduated from a 2-year college, vocational/associate degree*; 12 = *PhD, EDD, MD, DDS, LLB, LLD, JD, or other professional degree*. Descriptive statistics and further details on sample demographics are provided in Supplemental Table S1.

Health behaviors (i.e., smoking, alcohol, BMI), in addition to demographics, were controlled for in the next set of models. We indexed smoke pack years using the following questions: "Have you now or in the past used tobacco regularly?," "For how many years did you smoke regularly?," and "During this period, how many cigarettes did you smoke per day, on average?" Participants who never regularly smoked were given a value of zero (55% of sample). For the remaining participants, we multiplied the daily number of packs (number of cigarettes divided by 20) by the number of years as a regular smoker to index smoke pack years. We also controlled for alcohol use with two separate items. First, participants were asked, "During the past month, how often did you drink any alcoholic beverages, on the average?" on a 6-point scale (1 = *everyday*, 2 = *5 or 6 days a week*, 3 = *3 or 4 days a week*, 4 = *1 or 2 days a week*, 5 = *less than 1 day a week*, 6 = *never drinks*), which was reverse coded so that higher numbers represented greater frequency. Next participants were asked, "During the past month, on the days when you drank, about how many drinks did you drink on average?" These items are reported as "Alcohol Frequency" and "Avg No. of Drinks," respectively. Finally, participants self-reported their height and weight at Timepoint 2 (concurrent with the blood draw), allowing for a calculation of BMI. Descriptive statistics for all covariates are reported in Supplemental Table S1 and correlations between EAA, condition count, and health behaviors, are reported in Table 1.

# Results

## Associations Between Loneliness and EAA

Net of demographic covariates, there was a small, positive association between greater loneliness at Timepoint 1 and EAA at Timepoint 2 across each of the six measures ($0.04 \leq \beta \leq 0.11$). The association was statistically significant for DunedinPACE, GrimAge, and EAA average ($\beta = 0.11$, $p < .001$) and marginally significant for Horvath ($\beta = 0.08$, $p = .003$). See the top half of Table 2 for full model results. The addition of health behavior covariates slightly decreased the magnitude of the associations ($0.01 \leq \beta \leq 0.08$). The association remained marginally significant for GrimAge ($\beta = 0.08$, $p = .001$), but not Horvath ($\beta = 0.07$, $p = .01$), DunedinPACE ($\beta = 0.06$, $p = .02$), or EAA average ($\beta = 0.06$, $p = .02$). Demographic and health behavior covariates related to EAA in the expected directions. EAA was significantly predicted by male sex across four of the six measures ($-0.18 \leq \beta \leq -0.00$), lower levels of education in two ($-0.16 \leq \beta \leq 0.03$), greater smoke pack years across four ($0.02 \leq \beta \leq 0.45$), and higher BMI across three ($0.02 \leq \beta \leq 0.26$). Results were mixed for the race ($-0.09 \leq \beta \leq 0.25$) and alcohol-related ($-0.06 \leq \beta \leq 0.13$) covariates. The expected associations based on the previous literature (e.g., smoking, sex) were of greatest magnitude with the DunedinPACE and GrimAge variables. See the bottom half of Table 2 for full model results.

## Loneliness Predicting Change in Condition Count Mediated Through EAA

Net of demographic covariates, greater loneliness at Timepoint 1 was positively associated with residual increases in chronic condition count from Timepoint 1 to Timepoint 3 across models (six models were run corresponding to the included EAA variable). Associations were marginally significant ($0.12 \leq \beta \leq 0.13$, $.006 \leq p \leq .008$). The indirect associations between greater loneliness (mediated through the specified EAA variable) and residual increases in condition counts were nominally positive, not significant, and of smaller magnitude ($0.002 \leq \beta \leq 0.014$, $.03 \leq p \leq .63$). Indirect

**Table 2**
*Prediction of Epigenetic Age Acceleration by Loneliness*

| Model | Hannum acceleration | | | Horvath acceleration | | | PhenoAge acceleration | | | GrimAge acceleration | | | DunedinPACE | | | EAA average | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* |
| Model 1: Demographic covariates | | | | | | | | | | | | | | | | | | |
| Loneliness | 0.04 | 0.03 | .16 | *0.08* | *0.03* | *.003* | 0.04 | 0.03 | .20 | **0.11** | **0.03** | **<.001** | **0.11** | **0.02** | **<.001** | **0.11** | **0.03** | **<.001** |
| Chronological age | | | | | | | | | | | | | 0.06 | 0.03 | .01 | 0.03 | 0.03 | .38 |
| Sex (1 = female) | **−0.16** | **0.03** | **<.001** | **−0.13** | **0.03** | **<.001** | −0.04 | 0.03 | .17 | **−0.27** | **0.02** | **<.001** | *−0.08* | *0.03* | *.003* | **−0.19** | **0.03** | **<.001** |
| Race (1 = racially minoritized) | *−0.08* | *0.03* | *.008* | −0.00 | 0.03 | .91 | 0.03 | 0.03 | .29 | **0.16** | **0.03** | **<.001** | **0.28** | **0.03** | **<.001** | **0.11** | **0.03** | **<.001** |
| Education | 0.00 | 0.03 | .92 | 0.01 | 0.03 | .80 | −0.07 | 0.03 | .01 | **−0.26** | **0.03** | **<.001** | **−0.23** | **0.03** | **<.001** | **−0.16** | **0.03** | **<.001** |
| Model 2: Demographic and behavioral covariates | | | | | | | | | | | | | | | | | | |
| Loneliness | 0.03 | 0.03 | .24 | 0.07 | 0.03 | .01 | 0.01 | 0.03 | .69 | *0.08* | *0.02* | *.001* | 0.06 | 0.02 | .02 | 0.06 | 0.03 | .01 |
| Chronological age | | | | | | | | | | | | | 0.02 | 0.02 | .42 | −0.04 | 0.03 | .21 |
| Sex (1 = female) | **−0.16** | **0.03** | **<.001** | **−0.13** | **0.03** | **<.001** | −0.00 | 0.03 | .94 | **−0.18** | **0.02** | **<.001** | −0.04 | 0.02 | .12 | **−0.14** | **0.03** | **<.001** |
| Race (1 = racially minoritized) | *−0.09* | *0.03* | *.003* | −0.01 | 0.03 | .64 | 0.02 | 0.03 | .45 | **0.20** | **0.03** | **<.001** | **0.25** | **0.03** | **<.001** | **0.10** | **0.03** | **<.001** |
| Education | 0.01 | 0.03 | .67 | 0.03 | 0.03 | .45 | −0.03 | 0.03 | .37 | **−0.16** | **0.02** | **<.001** | **−0.13** | **0.03** | **<.001** | −0.08 | 0.03 | .01 |
| Smoke pack years | 0.02 | 0.03 | .47 | 0.03 | 0.03 | .24 | **0.14** | **0.03** | **<.001** | **0.45** | **0.03** | **<.001** | **0.25** | **0.03** | **<.001** | **0.27** | **0.03** | **<.001** |
| Avg. no. of drinks | 0.00 | 0.03 | .88 | 0.03 | 0.03 | .37 | 0.04 | 0.03 | .13 | *0.13* | *0.04* | *.001* | *0.13* | *0.04* | *.001* | *0.09* | *0.03* | *.009* |
| Alcohol frequency | 0.01 | 0.03 | .69 | −0.05 | 0.03 | .10 | 0.04 | 0.03 | .27 | 0.05 | 0.03 | .10 | −0.06 | 0.03 | .06 | −0.00 | 0.03 | .98 |
| BMI | 0.06 | 0.03 | .03 | 0.03 | 0.03 | .32 | **0.14** | **0.03** | **<.001** | 0.02 | 0.02 | .46 | **0.26** | **0.03** | **<.001** | **0.14** | **0.03** | **<.001** |

*Note.* β = standardized multiple regression coefficient. *p* = *p* value for multiple regression coefficient. Statistically significant ($p < .001$) effects are printed in bold font and marginally significant ($p < .01$) effects are printed in italics. Sex was self-reported as a binary variable (1 = female; 0 = male). Given small samples across groups, race was coded a binary variable (1 = racially minoritized, 0 = White/Caucasian). Education was coded as an ordinal variable ranging from 1 to 12 with higher values corresponding to categories of higher education levels (e.g., 12 = PhD, EDD, MD, DDS, LLB, LLD, JD, or other professional degree). Smoke pack years was calculated by multiplying self-reported daily cigarettes consumed (divided by 20) by the number of years reported as a regular smoker. Avg. no. of drinks was self-reported by asking participants their typical amount of alcohol consumption when they drink. Alcohol frequency was self-reported as an ordinal variable (e.g., 1 = *never drinks*; 3 = *3 or 4 days a week*; 6 = *everyday*). BMI was calculated using participant's self-reported heights and weights. Further details on variables are provided in Measures and Covariates Sections. EAA = epigenetic age acceleration; *SE* = standard error; BMI = body mass index.

associations were of greatest magnitude through DunedinPACE ($\beta = 0.012$, $p = .05$) and EAA average ($\beta = 0.014$, $p = .03$). EAA was also a nominally positive but nonsignificant predictor of increases in condition count ($0.02 \leq \beta \leq 0.11$), which was of greatest magnitude for EAA average ($\beta = 0.11$, $p = .01$) and DunedinPACE ($\beta = 0.09$, $p = .03$). See the top half of Table 3 for full model results. The associations remained similar in magnitude in the models that controlled for health behaviors for loneliness both directly ($0.11 \leq \beta \leq 0.12$, $.008 \leq p \leq .10$) and indirectly mediated through EAA ($-0.001 \leq \beta \leq 0.011$), as well as EAA directly ($-0.01 \leq \beta \leq 0.09$). See the bottom half of Table 3 for full model results.

## Testing Additional Potential Mediators

As a supplementary analysis, we considered models that evaluated whether associations between loneliness and residual change in chronic condition counts (net of demographic variables) were mediated by health behaviors, personality traits, and relational variables, rather than by EAA. None of the variables emerged as significant mediators; indirect associations with loneliness through the mediators ranged in magnitude from $-0.004 \leq \beta \leq 0.035$, with the largest effects through neuroticism ($\beta = 0.035$, $p = .05$) and BMI ($\beta = 0.12$, $p = .05$). Full results are reported in Supplemental Table S2.

## Change in Condition Count Predicted by the Loneliness by EAA Interaction

To test whether EAA moderated the association between loneliness and physical health, we considered models that included a statistical interaction term as a predictor of residual change in chronic condition counts. Net of loneliness, EAA, and demographic covariates, the EAA by loneliness interaction term was a nominally positive predictor of residual increases in condition counts across five of the six EAA variables ($-0.00 \leq \beta \leq 0.09$) and was marginally significant for DunedinPACE ($\beta = 0.09$, $p = .009$). See the top half Table 4 for full model results. The associations decreased in magnitude in the models that controlled for health behaviors ($-0.01 \leq \beta \leq 0.08$), remaining of greatest magnitude for DunedinPACE, though now falling shy of significance thresholds ($\beta = 0.08$, $p = .02$). In these models, greater loneliness ($0.10 \leq \beta \leq 0.12$, $.007 \leq p \leq .017$) and EAA ($-0.00 \leq \beta \leq 0.10$, $.02 \leq p \leq .94$) remained positive predictors of increased condition counts. See the bottom half of Table 4 full model results. Results are also summarized in Figure 1, which displays the prediction of residual change in condition counts by key constructs ($\beta$ weights for loneliness, all EAA measures, and loneliness by EAA) across covariate sets.

# Discussion

We aimed to examine whether self-reported loneliness related to accelerated epigenetic aging and the degree to which the two related to declining physical health in older adulthood. To do so, we tested whether EAA mediated and/or moderated the relationship between loneliness and residual change in chronic health condition counts. Further, we ran two sets of models with increasing covariate sets to consider the extent to which health behaviors like diet and exercise (BMI) and smoking attenuated the associations. We found evidence of a modest association between loneliness and accelerated aging approximately 2.5 years later that was slightly attenuated when accounting for health behaviors. Both greater loneliness and greater age acceleration were weak predictors of increased chronic condition count 7 years later. We did not find evidence consistent with DNA methylation mediating loneliness-disease associations. However, loneliness-disease associations were more pronounced for individuals with higher DunedinPACE EAA values, providing preliminary evidence of methylation as a moderator.

This evidence is consistent with the broad literature linking loneliness to multimorbidity and other adverse health outcomes (e.g., Hajek et al., 2020; O'Sullivan et al., 2022; Wang et al., 2023) and the more limited evidence that psychosocial adversities like loneliness relate specifically to accelerated epigenetic or biological aging (Beach et al., 2022; Galkin et al., 2022). Given the robust associations between loneliness and health, there has been interest in how loneliness "gets under the skin" to become biologically embedded and methylation has emerged as theoretically plausible (Freilich, 2023b). While there is preliminary evidence that EAA may mediate associations between loneliness and cognitive health (Lynch et al., 2023; Phillips, 2020), these results suggest mediation may not generalize to outcomes like multimorbidity. Indeed, although the indirect paths from loneliness to condition counts through EAA were nominally positive across measures, they were of small magnitude and shy of significance thresholds. The reason for this possible difference across outcomes is not known, though, speculatively, may involve a stronger link between loneliness and cognitive processes than with processes across other physiological systems. The multimorbidity dependent variable involves a range of conditions across systems, some of which may be unassociated with loneliness and some which may be linked with loneliness more through behavioral mediators, whereas some have theorized that not sufficiently engaging in social or cognitive activities may directly result in brain atrophy (Karska et al., 2023), or perhaps the associated epigenetic changes therein.

The moderation results were also inconclusive. The interaction between EAA and loneliness was modestly associated with increased condition count, though only met marginal significance thresholds in one measure (DunedinPACE) and without controlling for health behaviors. While far from conclusive, this preliminary evidence of moderation by DunedinPACE is consistent with loneliness and methylation having multiplicative impacts on health, wherein differences in methylation may affect biological responses to loneliness in a manner that increases individual disease vulnerability.

Evidence of mediation would be consistent Hawkley and Cacioppo's (2010) theory that loneliness is linked to physical health by causing hypervigilant physiological responses and the RNA transcript research (summarized by Cole, 2014) that suggests psychosocial adversities like loneliness can affect health through gene regulatory (epigenetic) pathways. Cole (2014) proposed that psychosocial adversities can be perceived in the central nervous system which leads to peripheral neural signaling and cellular signal transduction, causing transcription factor activation that regulates gene expression, which in turn alters inflammatory and immune responses and subsequent health. Similar pathways are plausible that lead to differential methylation, particularly given that transcription factors influence DNA methylation by recruiting DNA methyltransferases onto the genome as part of their response to receptor-mediated activation (Moore et al., 2013). In both cases, psychosocial adversities may become biologically embedded to have a chronic impact on health

**Table 3**

*Residual Change in Chronic Health Condition Count Predicted by Loneliness and Mediated Through Epigenetic Age Acceleration*

| Model | Hannum acceleration | | | Horvath acceleration | | | PhenoAge acceleration | | | GrimAge acceleration | | | DunedinPACE | | | EAA average | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* |
| Model 1: Demographic covariates | | | | | | | | | | | | | | | | | | |
| Loneliness direct effect | *0.12* | *0.05* | *.008* | *0.12* | *0.05* | *.007* | *0.13* | *0.05* | *.006* | *0.12* | *0.05* | *.008* | *0.12* | *0.05* | *.008* | *0.12* | *0.05* | *.008* |
| Loneliness indirect effect | 0.002 | 0.002 | .34 | 0.008 | 0.004 | .09 | 0.004 | 0.003 | .19 | 0.003 | 0.006 | .63 | 0.012 | 0.006 | .05 | 0.014 | 0.006 | .03 |
| EAA | 0.07 | 0.04 | .05 | 0.09 | 0.04 | .03 | 0.08 | 0.04 | .03 | 0.02 | 0.04 | .63 | 0.09 | 0.04 | .03 | 0.11 | 0.04 | .01 |
| Chronological age | 0.05 | 0.04 | .30 | 0.05 | 0.04 | .26 | 0.06 | 0.04 | .20 | 0.05 | 0.04 | .27 | 0.05 | 0.04 | .23 | 0.06 | 0.04 | .19 |
| Sex (1 = female) | 0.04 | 0.04 | .29 | 0.04 | 0.04 | .26 | 0.03 | 0.04 | .40 | 0.03 | 0.04 | .39 | 0.04 | 0.04 | .32 | 0.05 | 0.04 | .19 |
| Race (1 = racially minoritized) | 0.11 | 0.04 | .01 | 0.10 | 0.04 | .02 | 0.10 | 0.04 | .02 | 0.10 | 0.04 | .02 | 0.07 | 0.04 | .11 | 0.09 | 0.04 | .04 |
| Education | −0.04 | 0.04 | .29 | −0.05 | 0.04 | .24 | −0.04 | 0.04 | .29 | −0.04 | 0.04 | .38 | −0.03 | 0.04 | .48 | −0.03 | 0.04 | .42 |
| No. of chronic condition (T1) | **0.55** | **0.05** | **<.001** | **0.55** | **0.05** | **<.001** | **0.54** | **0.05** | **<.001** | **0.55** | **0.05** | **<.001** | **0.54** | **0.05** | **<.001** | **0.54** | **0.05** | **<.001** |
| Model 2: Demographic and behavioral covariates | | | | | | | | | | | | | | | | | | |
| Loneliness direct effect | 0.12 | 0.04 | .01 | *0.11* | *0.04* | *.009* | *0.12* | *0.05* | *.008* | *0.12* | *0.05* | *.009* | *0.12* | *0.05* | *.009* | *0.12* | *0.05* | *.009* |
| Loneliness indirect effect | 0.002 | 0.002 | .35 | 0.007 | 0.004 | .10 | 0.003 | 0.003 | .24 | −0.001 | 0.006 | .87 | 0.006 | 0.006 | .26 | 0.011 | 0.006 | .06 |
| EAA | 0.07 | 0.04 | .05 | 0.09 | 0.04 | .03 | 0.07 | 0.04 | .05 | −0.01 | 0.04 | .87 | 0.05 | 0.04 | .26 | 0.09 | 0.04 | .04 |
| Chronological age | 0.04 | 0.04 | .34 | 0.05 | 0.04 | .30 | 0.05 | 0.05 | .24 | 0.04 | 0.05 | .35 | 0.05 | 0.05 | .29 | 0.05 | 0.04 | .23 |
| Sex (1 = female) | 0.04 | 0.04 | .25 | 0.05 | 0.04 | .23 | 0.03 | 0.04 | .38 | 0.03 | 0.04 | .41 | 0.04 | 0.04 | .35 | 0.05 | 0.04 | .23 |
| Race (1 = racially minoritized) | 0.07 | 0.04 | .10 | 0.06 | 0.04 | .18 | 0.06 | 0.04 | .17 | 0.06 | 0.04 | .16 | 0.05 | 0.04 | .29 | 0.05 | 0.04 | .23 |
| Education | −0.02 | 0.04 | .62 | −0.02 | 0.04 | .55 | −0.02 | 0.04 | .60 | −0.02 | 0.04 | .63 | −0.02 | 0.04 | .70 | −0.02 | 0.04 | .70 |
| Smoke pack years | 0.05 | 0.03 | .14 | 0.05 | 0.03 | .14 | 0.04 | 0.03 | .22 | 0.05 | 0.03 | .14 | 0.04 | 0.03 | .28 | 0.03 | 0.03 | .40 |
| Avg. no. of drinks | 0.06 | 0.04 | .12 | 0.06 | 0.04 | .13 | 0.06 | 0.04 | .15 | 0.06 | 0.04 | .12 | 0.06 | 0.04 | .16 | 0.06 | 0.04 | .17 |
| Alcohol frequency | −0.09 | 0.04 | .03 | −0.08 | 0.04 | .03 | −0.09 | 0.04 | .03 | −0.08 | 0.04 | .04 | −0.08 | 0.04 | .04 | −0.09 | 0.04 | .03 |
| BMI | 0.09 | 0.04 | .04 | 0.10 | 0.04 | .03 | 0.09 | 0.04 | .05 | 0.09 | 0.04 | .03 | 0.08 | 0.04 | .06 | 0.09 | 0.04 | .05 |
| No. of chronic conditions (T1) | **0.54** | **0.05** | **<.001** | **0.53** | **0.05** | **<.001** | **0.53** | **0.05** | **<.001** | **0.54** | **0.05** | **<.001** | **0.53** | **0.05** | **<.001** | **0.53** | **0.05** | **<.001** |

*Note.* β = standardized multiple regression coefficient. *p* = *p* value for multiple regression coefficient. Statistically significant ($p < .001$) effects are printed in bold font and marginally significant ($p < .01$) effects are printed in italics. Sex was self-reported as a binary variable (1 = female; 0 = male). Given small samples across groups, race was coded a binary variable (1 = racially minoritized, 0 = White/Caucasian). Education was coded as an ordinal variable ranging from 1 to 12 with higher values corresponding to categories of higher education levels (e.g., 12 = PhD, EDD, MD, DDS, LLB, LLD, JD, or other professional degree). Smoke pack years was calculated by multiplying self-reported daily cigarettes consumed (divided by 20) by the number of years reported as a regular smoker. Avg. no. of drinks was self-reported by asking participants their typical amount of alcohol consumption when they drink. Alcohol frequency was self-reported as an ordinal variable (e.g., 1 = *never drinks*; 3 = *3 or 4 days a week*; 6 = *everyday*). BMI was calculated using participant's self-reported heights and weights. Further details on variables are provided in Measures and Covariates Sections. EAA = epigenetic age acceleration; *SE* = standard error; T1 = Time 1; BMI = body mass index.

**Table 4**

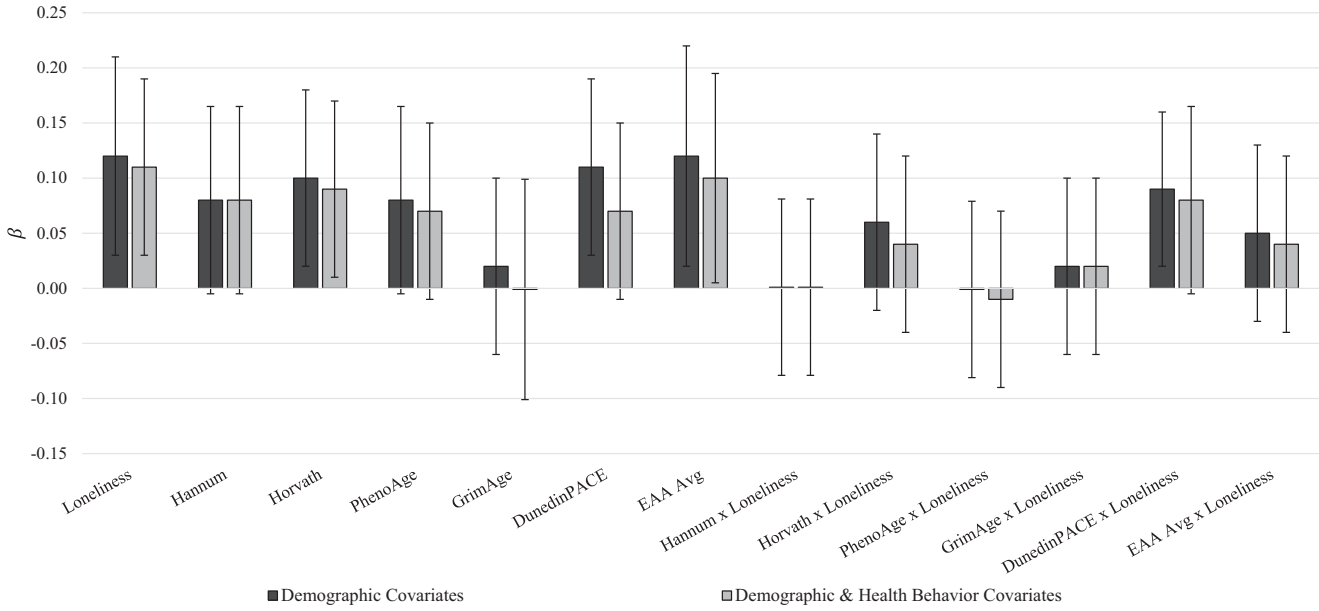
*Residual Change in Chronic Health Condition Count Predicted by the Epigenetic Age Acceleration by Loneliness Interaction*

| Model | Hannum acceleration | | | Horvath acceleration | | | PhenoAge acceleration | | | GrimAge acceleration | | | DunedinPACE | | | EAA average | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* | β | *SE* | *p* |
| Model 1: Demographic covariates | | | | | | | | | | | | | | | | | | |
| Loneliness × EAA | 0.00 | 0.04 | .98 | 0.06 | 0.04 | .16 | −0.00 | 0.04 | .96 | 0.02 | 0.04 | .53 | *0.09* | *0.03* | *.009* | 0.05 | 0.04 | .24 |
| Loneliness | *0.12* | *0.05* | *.008* | *0.12* | *0.04* | *.007* | *0.13* | *0.05* | *.006* | 0.12 | 0.05 | .01 | 0.10 | 0.04 | .02 | 0.11 | 0.04 | .01 |
| EAA | 0.08 | 0.04 | .08 | 0.10 | 0.04 | .03 | 0.08 | 0.04 | .05 | 0.02 | 0.04 | .60 | 0.11 | 0.04 | .01 | *0.12* | *0.05* | *.01* |
| Chronological age | 0.04 | 0.04 | .30 | 0.04 | 0.04 | .28 | 0.05 | 0.04 | .20 | 0.04 | 0.04 | .28 | 0.04 | 0.04 | .26 | 0.05 | 0.04 | .20 |
| Sex (1 = female) | 0.04 | 0.04 | .29 | 0.04 | 0.04 | .25 | 0.03 | 0.04 | .40 | 0.03 | 0.04 | .40 | 0.03 | 0.04 | .51 | 0.05 | 0.04 | .19 |
| Race (1 = racially minoritized) | 0.11 | 0.04 | .01 | 0.10 | 0.04 | .02 | 0.10 | 0.04 | .02 | 0.10 | 0.05 | .02 | 0.07 | 0.05 | .11 | 0.09 | 0.04 | .04 |
| Education | −0.05 | 0.04 | .29 | −0.05 | 0.04 | .22 | −0.04 | 0.04 | .29 | −0.04 | 0.05 | .36 | −0.03 | 0.04 | .49 | −0.04 | 0.04 | .39 |
| No. of chronic condition (T1) | **0.51** | **0.05** | **<.001** | **0.50** | **0.05** | **<.001** | **0.49** | **0.06** | **<.001** | **0.50** | **0.05** | **<.001** | **0.48** | **0.05** | **<.001** | **0.48** | **0.05** | **<.001** |
| Model 2: Demographic and behavioral covariates | | | | | | | | | | | | | | | | | | |
| Loneliness × EAA | 0.00 | 0.04 | .93 | 0.04 | 0.04 | .36 | −0.01 | 0.04 | .75 | 0.02 | 0.04 | .62 | 0.08 | 0.04 | .02 | 0.04 | 0.04 | .37 |
| Loneliness | 0.11 | 0.04 | .01 | 0.11 | 0.04 | .01 | *0.12* | *0.04* | *.007* | 0.11 | 0.04 | .01 | 0.10 | 0.04 | .02 | 0.11 | 0.04 | .01 |
| EAA | 0.08 | 0.04 | .08 | 0.09 | 0.04 | .03 | 0.07 | 0.04 | .09 | −0.00 | 0.05 | .94 | 0.07 | 0.04 | .12 | 0.10 | 0.05 | .02 |
| Chronological age | 0.04 | 0.04 | .34 | 0.04 | 0.04 | .32 | 0.05 | 0.04 | .24 | 0.04 | 0.04 | .36 | 0.04 | 0.04 | .36 | 0.05 | 0.04 | .23 |
| Sex (1 = female) | 0.05 | 0.04 | .25 | 0.05 | 0.04 | .23 | 0.03 | 0.04 | .39 | 0.03 | 0.04 | .43 | 0.03 | 0.04 | .52 | 0.04 | 0.04 | .26 |
| Race (1 = racially minoritized) | 0.07 | 0.05 | .10 | 0.06 | 0.05 | .17 | 0.06 | 0.04 | .17 | 0.06 | 0.05 | .16 | 0.05 | 0.05 | .30 | 0.05 | 0.04 | .24 |
| Education | −0.02 | 0.04 | .62 | −0.03 | 0.04 | .52 | −0.02 | 0.04 | .60 | −0.02 | 0.05 | .61 | −0.02 | 0.04 | .68 | −0.02 | 0.04 | .64 |
| Smoke pack years | 0.05 | 0.04 | .15 | 0.05 | 0.04 | .15 | 0.04 | 0.04 | .23 | 0.05 | 0.05 | .31 | 0.04 | 0.04 | .28 | 0.02 | 0.04 | .68 |
| Avg. no. of drinks | 0.07 | 0.05 | .13 | 0.06 | 0.04 | .16 | 0.07 | 0.04 | .13 | 0.07 | 0.04 | .14 | 0.05 | 0.04 | .23 | 0.06 | 0.05 | .22 |
| Alcohol frequency | −0.09 | 0.04 | .03 | −0.09 | 0.04 | .04 | −0.10 | 0.04 | .03 | −0.09 | 0.04 | .05 | −0.09 | 0.04 | .05 | −0.09 | 0.04 | .03 |
| BMI | 0.09 | 0.04 | .04 | 0.09 | 0.04 | .03 | 0.08 | 0.04 | .05 | 0.09 | 0.04 | .03 | 0.08 | 0.04 | .06 | 0.08 | 0.04 | .05 |
| No. of chronic conditions (T1) | **0.50** | **0.05** | **<.001** | **0.49** | **0.05** | **<.001** | **0.49** | **0.06** | **<.001** | **0.49** | **0.05** | **<.001** | **0.48** | **0.05** | **<.001** | **0.48** | **0.05** | **<.001** |

*Note.* β = standardized multiple regression coefficient. *p* = *p* value for multiple regression coefficient. Statistically significant ($p < .001$) effects are printed in bold font and marginally significant ($p < .01$) effects are printed in italics. Sex was self-reported as a binary variable (1 = female; 0 = male). Given small samples across groups, race was coded a binary variable (1 = racially minoritized, 0 = White/Caucasian). Education was coded as an ordinal variable ranging from 1 to 12 with higher values corresponding to categories of higher education levels (e.g., 12 = PhD, EDD, MD, DDS, LLB, LLD, JD, or other professional degree). Smoke pack years was calculated by multiplying self-reported daily cigarettes consumed (divided by 20) by the number of years reported as a regular smoker. Avg no. of drinks was self-reported by asking participants their typical amount of alcohol consumption when they drink. Alcohol frequency was self-reported as an ordinal variable (e.g., 1 = *never drinks*; 3 = *3 or 4 days a week*; 6 = *everyday*). BMI was calculated using participant's self-reported heights and weights. Further details on variables are provided in Measures and Covariates Sections. EAA = epigenetic age acceleration; *SE* = standard error; BMI = body mass index; T1 = Time 1.

**Figure 1**

*Prediction of Residual Change in Chronic Conditions by Loneliness, Epigenetic Age Acceleration, and Their Interaction Across Models*




*Note.* Standardized β weights from multiple linear regressions reported to show covariate-adjusted associations with residual change in number of chronic conditions. Analyses adjusted progressively for demographics (left) and then demographics and health behaviors (right). Error bars correspond to two standard errors. EAA Avg = epigenetic age acceleartion average; composite average of other five measures.

via their impact on gene expression. Although none of the mediation paths were statistically significant, they were nominally positive across measures, so mediation requires further study in larger samples, particularly with later generation measures like DunedinPACE.

The moderation analyses also yielded small but nominally positive interactive effects that were of greatest magnitude for DunedinPACE, so they similarly would benefit from additional research. It may be the case that loneliness and EAA operate synergistically wherein loneliness is more strongly linked to adverse health in the presence of an age accelerated methylation profile, but that the impact of loneliness on health conditions does not occur through DNA methylation. Nonetheless, individual differences in loneliness are of broad public health importance, and more research will be necessary to understand when and how common emotional experiences of this kind impact our physical health.

Finally, we tested five distinct EAA variables in each of these models (and a composite), and the later generation DunedinPACE and GrimAge measures tended to have the strongest associations with known demographic and health correlates (e.g., sex, education, smoking, BMI). This is consistent with evidence that newer clocks trained on health indicators beyond chronological age tend to have stronger associations with a range of variables than the first-generation clocks trained only on age (Oblak et al., 2021). Notably, DunedinPACE and GrimAge also had the largest associations with loneliness, and DunedinPACE was one of the stronger predictors of chronic conditions both independently and interactively with loneliness.

## Limitations

Additional theoretical mechanisms by which loneliness becomes biologically embedded include health-limiting behaviors, demographic confounding, and genetic overlap. Evidence to date suggests subtle and perhaps interactive impacts from each these effects, rather than a singular, primary mechanism (Cacioppo et al., 2002; Hawkley & Cacioppo, 2010). We aimed to statistically control for the first two possibilities, though the degree to which overlapping genetic architectures may account for the observed associations is unknown.

Another clear limitation is that EAA and loneliness were only measured at one timepoint, so conclusions about temporal sequencing cannot be made. Though loneliness was measured before EAA, reverse causation—faster aging impacting an individual's social functioning and experience of loneliness—is indeed plausible and cannot be ruled out. The measurement of chronic conditions at two timepoints, both before and after the EAA measurement is a strength of the present study, though a large portion of the sample was not yet assessed in the later timepoint, yielding a relatively modest sample size in the longitudinal analyses. Further, data from the Core and Refresher samples were collected at different times, which may present concerns about sample heterogeneity. In addition, both loneliness and number of chronic conditions were self-reported, raising the possibility that evaluative consistency bias may artificially inflate associations. Methodologically, the practice of partialing a variable on confounders to remove extraneous variance (i.e., effect of loneliness on EAA net of health behaviors) creates difficulty in interpreting the partialed variable (Hoyle et al., 2023) and in separating the effects of "covariates" from those of meaningful "predictors" in the measurement model.

Though frequently used as a measure of generalized health that is common in older adults, condition count is just one indicator and may not be representative of loneliness' broad impacts on health as it is interpreted herein. Future studies should additionally consider the subjective burden of diseases, as well as other health outcomes to enhance our understanding of the impacts of loneliness. It is unclear

how results would generalize to younger adult and adolescent samples. Future studies would benefit from sampling across the lifespan to understand the course of associations between loneliness and biological aging. Finally, this study analyzed DNA methylation in circulating blood, and it is unclear whether similar effects would take place in other tissues, or how the known variability in the cellular composition of circulating blood (and its consequences for whole blood DNA methylation profiles; Jaffe & Irizarry, 2014) affects the present findings. Future research that directly measures circulating blood composition will be required to separate per-cell differences in epigenetic age from the effects of age on changing blood cell distributions, and to determine their respective relations to loneliness and its health correlates.

## Conclusion

We aimed to evaluate whether epigenetic influences on DNA methylation could help explain loneliness-related health risk from midlife to older adulthood. Loneliness was weakly associated with accelerated epigenetic aging and they both predicted increases in multimorbidity. In the sample, EAA did not significantly mediate the loneliness-morbidity association, though the effect was more pronounced for those with higher DunedinPACE EAA values. This suggests that the impacts of loneliness on health may not occur through DNA methylation; however, those impacts may be more extensive in the presence of an age accelerated methylation profile. Overall, DNA methylation is a promising possibility, but conclusions about its role in the relationship between loneliness and physical health will require future inquiry to define whether it acts as a mediator through which associations occur, a moderator magnifying loneliness's impact, or a correlated but not mechanistically involved variable.

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