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Neighborhood Disadvantage and Biological Aging: Using Marginal Structural Models to Assess the Link Between Neighborhood Census Variables and Epigenetic Aging

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Abstract

Objectives: Past research has reported an association between neighborhood disadvantage and healthy aging, but most of these studies utilize self-report measures of health or physical functioning and do not properly account for neighborhood selection effects, creating concerns regarding inflated associations. To overcome these limitations and provide a more stringent estimate of effects, the current study investigated the effect of neighborhood disadvantage on aging using newly developed epigenetic methods to assess rate of biological aging and marginal structural modeling (MSM) to account for potential confounds due to neighborhood selection.

Methods: We tested the hypothesis that neighborhood disadvantage accelerates aging using U.S. census data and five waves of interview data from a sample of 100 middle-aged African American women. Using a recently developed epigenetic index of aging, biological age was measured using weighted methylation values at 71 CpG sites. We calculated a measure of accelerated methylomic aging (in years) based upon the residual scores resulting from a regression of methylomic age on chronological age.

Results: Controlling for a variety of individual difference factors that could be confounded with neighborhood effects, including various health behaviors, neighborhood disadvantage was associated with accelerated biological aging. Using MSM to account for selection effects, a standard deviation increase in neighborhood disadvantage accelerated aging an average of 9 months.

Conclusions: Our findings converge with prior work to provide strong evidence that neighborhood context is a significant determinant of healthy aging.

Keywords: Aging—Biological age—Marginal structural modeling—Neighborhood—Selection bias

Over the past two decades, numerous studies have reported an association between living in a disadvantaged neighborhood and a range of negative physiological consequences (Beard et al., 2009; Bosma, van de Mheen, Borsboom, & Mackenbach, 2001; York Cornwell & Cagney, 2014). Although this research suggests that neighborhood context likely plays a pivotal role in health disparities and the aging process, the studies to date have been plagued by methodological issues that remain to be addressed. First, most studies employ self-report measures of both neighborhood context and aging. Thus, the associations reported in previous research are vulnerable to concerns about inflated or deflated associations due to measurement error. Second, critics of this research have argued that people may select themselves into neighborhoods, leading neighborhood to be confounded with a range of

individual difference variables. The present study attempts to avoid these limitations by using Census data to assess neighborhood disadvantage and a recently validated DNA methylation index to assess accelerated biological aging. Further, we utilize marginal structural modeling (MSM) to adjust for potential neighborhood selection effects resulting in a test of neighborhood effects on health that is not dependent on self-report and that controls for a range of confounds.

Neighborhood Disadvantage and Aging

A long history of research has indicated that neighborhood disadvantage increases morbidity and mortality risks (Bosma et al., 2001). This relationship appears to be due to the fact that individuals residing in such neighborhoods often perceive that people in the area cannot be trusted, feel powerless, struggle with financial hardships, and believe that life is essentially chaotic (Ross, 2011). Thus, prolonged involvement with such environments provides highly salient cues of threat that trigger physiological stress, negative emotionality, and a cascade of biological responses that, over time, cause wear and tear on physiological systems and increased risk of premature aging (Simons et al., 2016).

Compared to members of other racial groups, many African Americans live in disadvantaged neighborhoods (Peterson & Krivo, 2010). Despite moving, they often still reside in economically disadvantaged areas. They are what Wilson (1987) has labeled, the "truly disadvantaged." Building on this idea, we expect that neighborhood effects for African Americans operate as chronic stressors lasting for years or a lifetime even controlling for residential mobility.

Methylomic Age as an Objective Measure of Accelerated Aging

Prior health-related research has often relied on self-report measures of health status and physical functioning. Unfortunately, such measures often suffer from measurement error and social desirability that may serves to under- or over-estimated (Altman, Van Hook, & Hillemeier, 2016; Benitez-Silva, Buchinsky, Man Chan, Cheidvasser, & Rust, 2004). Since the 1960s, however, researchers have been aware that there is a strong association between age and DNA methylation (Jones, Goodman, & Kobor, 2015; Weidner & Wagner, 2014). Based upon this finding, Hannum and colleagues (2013) recently construct an index that quantifies level of DNA methylation in regions found to be highly reliable markers of cellular-level aging. Using blood leukocytes, Hannum and colleagues (2013) identified 71 CpG sites scattered throughout the human genome where methylation levels correlate strongly with chronological age. The correlation between age and the weighted sum of the methylation scores at these 71 sites correlated over .90 in the samples used to develop the measure and between .82 and .85 in subsequent studies using this instrument (Beach et al., 2015). Indeed, chronological age is much more strongly related to methylation-based aging than to telomere length or other biological markers of aging (e.g., Horvath 2013; Marioni et al., 2015; Wolf et al., 2016; Zheng et al., 2016), and this may be especially true when the sample is African American, for whom telomere length may be longer than for Whites (Diez Roux et al., 2009), and for whom some researchers have reported positive associations with stress (Boks et al., 2015). Many of the markers included in the Hannum and colleagues (2013) index are within or near genes with known functions in aging-related conditions, including Alzheimer's disease, cancer, DNA damage, tissue degradation, and oxidative stress. After age 20, there appears to be a rather constant rate of methylation change in the 71 sites identified by Hannum and colleagues (2013). Thus, they can be used as a "biological clock" to assess, at any point during adulthood, the extent to which an individual is experiencing accelerated or decelerated biological aging (Hannum et al., 2013).

Recent evidence suggests that biological aging can be influenced by environmental conditions throughout the life span. Simons et al. (2016), for example, recently reported a robust relationship between economic hardship and the Hannum index of accelerated aging that remained significant even after controlling for a variety of health-related behaviors. Neighborhood studies have presented evidence that for people living in disadvantaged neighborhoods, ambient threat, social strain, social isolation, disrespect, and hopelessness, promote physiological distress which, in turn, influences health-related outcomes (Ross, 2011). These various findings suggest that long-term living in neighborhoods characterized by concentrated disadvantage likely accelerates biological aging or what Geronimus (2013) has labeled "biological weathering." This hypothesis is tested in the present study.

Marginal Structural Modeling

Individual characteristics may confound the relationship between neighborhood disadvantage and individual well-being. This potential problem is usually referred to as the issue of self-selection bias, but encompasses all individual differences that could confound the effect of an independent variable when it is studied in the absence of random assignment to level of exposure. This selection process is complex as it is affected by time-varying individual characteristics (Sampson, 2012). Previous studies have attempted to reduce this bias by controlling for baseline neighborhood status and other potentially confounding variables. Simply incorporating control variables into a traditional regression model, however, has been criticized for being inadequate to control for confounding variables that vary over time and are affected by previous treatment (Robins, 2000). In particular, traditional regression models ignore

the fact that reciprocal cause-and-effect relationships exist between exposures and covariates. For example, as shown in Figure 1, the effects of baseline covariates predict neighborhood disadvantage at baseline which, in turn, predict both covariates and neighborhood disadvantage at follow-up. Covariates at baseline may also have indirect effects on neighborhood disadvantage at follow-up through covariates at follow-up.

MSM offers a significant advantage over traditional models when controlling for time-varying confounders affected by previous treatment. This method uses the inverse probability of treatment weighting (IPTW) to balance previous treatment and confounding factors across treatment groups at each time period. Based upon this idea, the weighting creates a pseudo-population in which there is no association between an exposure (e.g., neighborhood disadvantage) and covariates (e.g., individual socioeconomic measures), but the effect of an exposure on an outcome is the same as in the actual study population. Simulation studies (e.g., Havercroft & Didelez, 2012) have shown that MSM not only provides more accurate estimates of time-varying measures than traditional regression models, but also makes causal inference possible with observational data if the assumptions of exchangeability (i.e., no unobserved confounding), positivity (i.e., positive probability of exposure at every level of observed confounders), consistency (i.e., well-defined exposure), and correct model specification are met.

Using MSM and the biological clock developed by Hannum and colleagues (2013), the current study tested the hypothesis that residing in a disadvantaged neighborhood is associated with accelerated methylomic aging, even after adjusting for neighborhood disadvantage and potential confounders at a previous time point.

Method

Sample

We tested this hypothesis using data from the five waves of the Family and Community Health Study (FACHS). FACHS is a longitudinal study of several hundred African American families that was initiated in 1997. Details regarding recruitment are described by Simons et al. (2016). At the first wave (1997–1998), the FACHS sample consists of 889 African American children and their primary caregivers (PCs; 829 women and 60 men). The second, third, fourth, and fifth waves of data were collected from 1999 to 2000, 2001 to 2002, 2004 to 2005, and 2007 to 2008 to capture information when target youths were ages 12 to 13, 14 to 15, 17 to 18 and 20 to 21 years, respectively. Of the 889 PCs interviewed at Wave 1, 693 were interviewed again at Wave 5 (77.26% of the original sample).

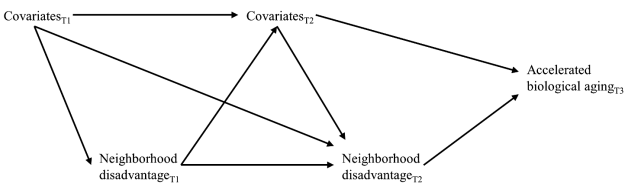

Figure 1. Casual models linking covariates, neighborhood disadvantage, and accelerated biological aging.

At Wave 5, using only those identified as being of African American descent, 100 women were randomly selected from the roster of PCs to participate in an epigenetic assessment. Due to the costs associated with the blood draws and epigenetic assays, the use of a subsample was necessary. Studies have indicated that population genetic admixture may confound genetic effects. We employed the Structure program, version 2.3.4 (Falush, Stephens, & Pritchard, 2007) with a panel of 24 ancestry informative markers to estimate an ancestry proportion of each participant. The average proportion of African ancestry in our sample is 94.7% (Lei, Simons, Edmond, Simons, & Cutrona, 2014). There were no missing values at waves 4 and 5 on any of the study variables. We used the last observation carried forward approach for imputing missing values at waves 1, 2, and 3. Comparisons of this subsample with those who were not included in the methylation assessment did not reveal any significant differences with regard to either demographic characteristics or health-related variables (see Supplementary Table S1).

Procedures

Informed consent was obtained from participants and all study procedures were approved by the University institutional review board. Computer-assisted interviews were administered in the respondent's home and took on average about two hours to complete. The instruments were presented on laptop computers. Questions appeared in sequence on the screen, which both the researcher and participant could see. The researcher read each question aloud and the participant entered an anonymous response using a separate keypad.

In addition, participants were also asked to provide a blood sample at Wave 5. A certified phlebotomist drew four tubes of blood (30 ml) from each participant and shipped it the same day to a laboratory at the University of Iowa for preparation. Upon receipt, the blood tubes were inspected to ensure anticoagulation and aliquots of blood were diluted 1:1 with phosphate buffered saline pH 8.0. Mononuclear cell pellets were separated from the diluted blood specimen by centrifugation with ficoll (400 g, 30 min) and the mononuclear cell layer was removed from the tube using a transfer pipette, resuspended in a phosphate buffered saline solution, and briefly centrifuged again. The resulting cell pellet was resuspended in a 10% DMSO/RPMI solution and frozen at −80 degrees Celsius until use. Genomic DNA was extracted with Qiagen DNA Mini Kits, and quality verified on an Agilent

2100 Bioanalyzer. Typical DNA yield for each pellet was between 10 and 15 µg of DNA.

Methylation Procedures

Genotyping was conducted with the Illumina Infinium (Sequenom, Inc., San Diego, CA) HumanMethylation450 Beadchip. Participants were randomly assigned to 16 sample "slides/chips" with groups of eight slides being bisulfite converted in a single plate, resulting in two "batches/plates." A replicated sample of DNA was included in each plate to aid in assessment of batch variation and to ensure correct handling of specimens. The replicate sample was examined for average correlation of beta values between plates and was found to be greater than .99. Using the ChAMP pipeline for quality control and normalization (Morris et al., 2014), data were filtered based on these criteria: (a) probes with the detection p value >0.01 in more than one sample; (b) probes with the beadcount <3 in at least 5% of the samples; (c) probes containing SNP sites; and probes on the X or Y chromosome. This resulted in a data set of 433,188 informative CpG sites. Finally, the remaining probes were normalized for adjusted Type I and Type II assays using Beta-Mixture Quantile (BMIQ) normalization method (Teschendorff et al., 2013). The results showed that there were no significant batch or chip effects after quantile normalization (see Supplementary Figure S1). Details regarding processing and preparation of the methylation data are described by Simons and Colleagues (2016).

Measures

Accelerated methylomic aging

Peripheral blood mononuclear cells (PBMC) methylomic age was calculated following the method proposed by Hannum and colleagues (2013). Using peripheral blood from several large cohorts of adults and penalized regression models and subsequent bootstrap analyses, Hannum and colleagues (2013) identified 71 CpG sites scattered throughout the human genome where methylation levels correlate strongly with chronological age. In the current study, the measure of PBMC methylomic age is based upon the weighted sum of methylation values at these 71 CpG sites (Hannum et al, 2013). Finally, we formulated a measure of accelerated aging using the unstandardized residual scores from the regression of PBMC methylomic age on chronological age (Marioni et al., 2015; Wolf et al., 2016; Zheng et al., 2016). These residuals had a mean of zero and represented both positive and negative deviations from chronological age (in years), with positive scores indicating accelerated aging. Consistent with previous studies (Marioni et al., 2015; Zheng et al., 2016), accelerated aging was significantly correlated (r = .199, p = .047) with self-reported chronic illness (see Supplementary Table S2).

Cell-type composition

To adjust for cellular heterogeneity that can affect methylation-based scores, we controlled for cell-type distribution in the models. Cell-type composition was estimated using the "EstimateCellCounts" function in the minfi Bioconductor package, which is based on the reference-based method developed by Houseman and colleagues (2012). Using this approach, we estimated cell-type proportions in whole blood for CD4+ T cells, CD8+ T cells, Natural Killer cells, B cells, and monocytes.

Neighborhood disadvantage

At waves 4 and 5, the measure of neighborhood disadvantage was created using the data from the U.S. Census Bureau's American Community Survey 5-Year Estimates (2006–2010), which was mapped onto the geocodes for our study participants' residential addresses in 2004 and 2007. At waves 1 and 3, neighborhood disadvantage was assessed with the 2,000 census Summary Tape File 3A, which was geocoded with participants' residential addresses in 1997, 1999, and 2001. We formed a scale of neighborhood disadvantage using five census-tract items: median household income (reverse coded), percent unemployed males, percent below the poverty threshold, percent who are single-mother families, and percent receiving public assistance. The mean and standard deviations among these five indicators are presented in Supplementary Table S3. The five items were standardized and summed. A higher score represented a more disadvantaged neighborhood. Across all five waves of data collection, 22% never moved, 58% moved one or two times, and 20% of respondents moved three or more times, and the average number of moves was 1.48 (SD = 1.16).

Covariates

To isolate the effect of neighborhood disadvantage per se, analyses were controlled for a comprehensive set of time-varying covariates at waves 1–5. Sociodemographic covariates included *family per capita income*, *employment status* (employed = 1), *receives welfare* (yes = 1), the total *number of children* in the household, and *residential relocation* (yes = 1). "Educational attainment" was measured as dummy variables indicating respondents with less than a high school diploma, a high school diploma, or a graduate equivalent degree (GED), some college, or at least college degree. "Marital status" was measured by dummy variables for single, cohabiting, or married. "Health insurance" was measured dichotomously (yes = 1, no = 0).

We also included health-related covariates at waves 1–5. Thus, current status of the covariates was measured at the time of the blood draw (Wave 5). Two items indexed substance use: *binge drinking* and *cigarette use*. Respondents reported whether during the prior 12 months they had smoked cigarettes (yes = 1, no = 0); had three or more drinks of alcohol, ranging from 0 (never) to 5 (every day). "Healthy diet" was assessed using two items that asked about frequency of fruit and vegetable consumption

during the previous 7 days. The relationship between the two items was significant (r = .237, p = .016). Responses ranged from 0 (none) to 4 (twice a day or more) and were averaged to form the healthy diet variable. "Exercise" was measured with two items: On how many of the past 7 days did you exercise or participate in physical activity for at least 30 min that made you breathe hard such as running or riding a bicycle hard? And, on how many of the past 7 days did you exercise or participate in physical activity for at least 30 min that did not make you breathe hard, but was still exercise such as fast walking, slow bicycling, skating, pushing a lawn mower, or doing active household chores? The response categories ranged from 0 (0 days) to 4 (all 7 days). These two items were correlated (e.g., Wave 5: r = .419, p < .001). Scores on the two items were averaged to form the exercise measure.

Analytic Strategy

For all analyses, we used STATA 14 (StataCorp, 2015). Given that more than 70% of census tracts had only one participant and there was no significant intra-class correlation for accelerated aging, this study did not use multilevel modeling. To avoid overestimating the results, parameters in our models are examined using maximum likelihood estimate with robust standard errors. We begin by examining OLS regression models with robust standard errors using data at the time of the blood draw. This method is comparable to previous cross-sectional neighborhood studies. Next, we will run a mediating model to investigate the impact of cumulative neighborhood disadvantage on individual differences in rate of aging. And, given that respondents were not randomly assigned to residential locations, we will then run MSM and IPTW using five waves of data to assess the relationship between neighborhood disadvantage and aging. Because neighborhood disadvantage is a continuous measure, conditional densities were used and estimated through linear regression (Robins, 2000). Let N_i denote neighborhood disadvantage and C_i be a set of time-varying and invariant covariates. The IPT weights are given by

$$w_i = \prod_{t=1}^{t} \frac{1}{f[N_{it} \mid N_{i(t-1)}, C_{i(t-1)}]}, \quad (1)$$

For individual i, the IPT weighting calculates conditional densities of exposure to disadvantaged neighborhoods at time t, conditional on the set of neighborhood disadvantage and covariates at t–1. However, the weight (w_i) would have infinite variance. Hernán, Brumback, & Robins (2000) suggest using stabilized IPT weights to reduce bias:

$$sw_i = \prod_{t=1}^{t} \frac{f[N_{it} \mid N_{i(t-1)}]}{f[N_{it} \mid N_{i(t-1)}, C_{i(t-1)}]}, \quad (2)$$

where stabilized weights (sw_i) has the same denominator as in Equation 1. The numerator of the stabilized weights (sw_i) is conditional densities of exposure to a disadvantaged neighborhood given neighborhood disadvantage at the previous time point. Finally, pooled regression models are weighted by the stabilized IPT weights. Therefore, individuals who are underrepresented in exposure assignment are given proportionately higher weights, whereas those who are highly represented in exposure assignment are given proportionally lower weights. Sample STATA code related to the stabilized IPT weights is provided in Supplementary Appendix 1.

Results

Initial Findings

At the time of the blood draw, the mean chronological age of the respondents was 48.52 years (SD = 9.30). As expected, methylomic age was strongly associated with chronological age (r =.815, p = 5.964e–25, see Figure 2), and all but five of the 71 CpGs showed a significant association with chronological age in our sample (see Supplementary Table S4). Mean ages predicted by the Hannum's methylomic index were 1.22 years higher than the actual chronological age of the sample. Fifty-four percent of respondents had a methylomic age greater than their chronological age. This indicates a tendency in the sample toward accelerated aging.

The zero order correlations among the study variables are presented in Supplementary Table S5. As hypothesized, although the strength of the relationship was low to moderate given the relative homogeneity of the sample, there was a significant relationship between neighborhood disadvantage and per capita income (r = –.287, p = .004). In addition, these two variables were significantly associated with accelerated aging estimates residualized for chronological age (r = .291, p = .003 and r = –.290, p = .003, respectively).

OLS Regression Modeling

The G*Power program was used to assess the extent to which we had sufficient power to detect neighborhood

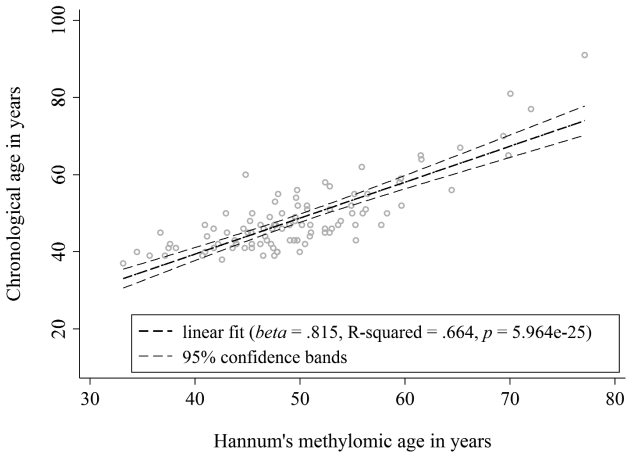

Figure 2. The relationship between methylomic age and chronological age.

effects with 100 participants. The program indicated a statistical power greater than 80% at $\alpha = .05$ for the model used in our regression analysis presented below (effect size of $\eta^2 = .084$). Further, regression diagnostics revealed that assumptions for normality (Shapiro–Wilk test: $W = .989$, $p = .551$) and homoscedasticity (Breusch–Pagan/Cook–Weisberg test $\chi^2_{(1)} = .040$, $p = .832$) were met.

The results of the analysis using regression models with robust standard errors are presented in Table 1. Model 1 shows that the main effect for neighborhood disadvantage is significant ($b = 1.370$, $p = .002$), and that a standard deviation increase in neighborhood disadvantage is association with 1.370 years increase in methylomic age. In other word, individuals living in disadvantaged neighborhoods show significantly accelerated aging.

The analysis presented in Model 2 added covariates and cell-type composition at Wave 5. As hypothesized, with the covariates and cell-type variation controlled, the results remain significant and in the expected direction ($b = 0.962$, $p = .018$). The last column of Table 1 includes a squared term for neighborhood disadvantage as a test of potential nonlinear effects. This term is not statistically significant ($b = -0.065$, *ns*), indicating the absence of any substantial nonlinear effect. To insure robustness of effects, we repeated the analyses controlling for health-related covariates by summing across Waves 1–5. The results showed no change in the pattern of effects (see Supplementary Table S6).

Mediating Model

Next, we ran a mediating model to determine whether cumulative exposure to neighborhood disadvantage is more important than exposure during any particular

Table 1. Robust Regression Models Examining Neighborhood Disadvantage as an Independent Variable of Biological Aging

	Model 1			Model 2			Model 3		
	b	*SE*	*p* value	*b*	*SE*	*p* value	*b*	*SE*	*p* value
Neighborhood disadvantage	1.370**	0.434	.002	0.962*	0.398	.018	0.965*	0.399	.018
Neighborhood disadvantage squared							−0.065	0.273	.811
Per capita income				−0.190	0.514	.712	−0.188	0.516	.717
Education (high school is reference)									
Less than a high school education				0.407	1.093	.711	0.406	1.102	.714
Some college				0.557	0.907	.541	0.577	0.934	.539
At least a college degree				−1.621	1.511	.286	−1.620	1.523	.291
Marital status (single is reference)									
Married				−0.529	1.053	.617	−0.527	1.060	.620
Cohabited				0.118	1.066	.912	0.140	1.080	.897
Employment status				1.652*	0.827	.049	1.638†	0.830	.052
Receives welfare				−1.064	1.979	.592	−1.089	2.005	.589
Number of children in household				−0.025	0.254	.921	−0.024	0.255	.926
Number of relocation from W1 to W5				−0.143	0.399	.722	−0.127	0.414	.759
Health insurance				−1.984*	0.987	.048	−1.968†	0.993	.051
Current cigarette use				0.989	0.839	.242	1.011	0.840	.233
Current binge drinking				−0.883	1.386	.526	−0.897	1.393	.522
Current healthy diet				0.595	0.492	.230	0.601	0.499	.231
Current exercise				0.132	0.324	.684	0.126	0.330	.705
CD8+ T cells				−32.892**	7.915	.000	−32.810**	8.060	.000
CD4+ T cells				−13.691**	4.998	.008	−13.675**	5.054	.008
Natural killer cells				17.914*	6.989	.012	17.781*	7.027	.013
B cells				−4.647	5.405	.393	−4.679	5.426	.391
Monocytes				−7.759	5.960	.197	−7.711	6.043	.206
Constant	0.000	0.453	1.000	7.184*	3.296	.032	7.193*	3.303	.032
R^2	0.085			0.525			0.525		

Note: Unstandardized (*b*) coefficients shown with robust standard errors in parentheses; neighborhood disadvantage and per capita income are standardized by z-transformation (mean = 0 and *SD* =1). $N = 100$. $^{\dagger}p \le .10$, $^{*}p \le .05$, $^{**}p \le .01$ (two-tailed tests).

stage of life. As Figure 3 shows, the association between neighborhood disadvantage (averaged across waves 1 and 2) and neighborhood disadvantage (averaged across waves 3 and 4) was significant (β = .673, $p < .001$), the relationship between neighborhood disadvantage (averaged across waves 3 and 4) and neighborhood disadvantage at Wave 5 was significant (β = .389, $p < .001$), and the association between neighborhood disadvantage at Wave 5 and accelerated aging was also significant (β = .237, $p < .023$). Using a bootstrapping technique with 1,000 replications, the results indicated that the mediating effect of early neighborhood disadvantage on accelerated aging through both neighborhood disadvantage (averaged across waves 3 and 4) and current neighborhood disadvantage was significant (indirect effect = .033, 95% confidence interval [0.004, 0.107]). The results revealed that it is the cumulative effect of neighborhood disadvantage that accelerates biological aging.

Marginal Structural Modeling

Given the presence of significant cumulative effect of neighborhood disadvantage, we used MSM to adjust for time-invariant and time-varying covariates thought IPTW. Prior to running MSM, we ran the unadjusted and adjusted pooled regressions on five waves of data. As can be seen in Table 2, without controlling for covariates, the main effect for neighborhood disadvantage is significant in Model 1 ($b = 1.002$, $p = .006$). Model 2 adds sociodemographic and health-related covariates. As expected, the relationship between neighborhood disadvantage and accelerated aging remains statistically significant ($b = 0.671$, $p = .004$).

Next, the IPTW was calculated using regression models after adjustment for all time-invariant and time-varying covariates shown in Supplementary Table S7. To avoid an extreme variation of weights, we first checked the variance of IPT weights. Unstabilized IPTW (w_i) were associated with substantial variability ($\delta^2 = 4598.823$), whereas this variability was eliminated by using stabilized IPTW ($\delta^2 = 0.016$). Since extreme weights can bias the standard error estimates (Hernán, Brumback, & Robins, 2000), stabilized IPTW are considered to be the most appropriate approach for adjusting our models.

As shown in Model 3 of Table 2, neighborhood disadvantage continues to be a significant exposure of biological aging ($b = 0.903$, $p = .011$) after adjusting for the time-varying and time-invariant covariates through stabilized IPT weights. Controlling for time-invariant covariates (e.g., number of relocations and cell types) in a pooled regression with stabilized IPTW, Model 4 shows a pattern of findings that are very similar to those found for models 1 to 3. The effect in Model 4 is stronger in magnitude than for conventional adjusted regression models. The finding suggests that a standard deviation increase in neighborhood disadvantage is associated with .752 years increase in methylomic age. Supplementary Figure 2 depicted this effect. As can be seen, the regression line crosses the line of deviation of methylomic age from chronological age at zero, suggesting that individuals living in disadvantaged neighborhoods show significantly accelerated aging whereas those living in advantaged neighborhoods demonstrate significantly decelerated aging.

Taken together, the substantive findings across these models are entirely consistent with the results showed in Table 1 and Figure 3. This pattern of findings supports our hypothesis that long-term exposure to disadvantaged neighborhoods is associated with accelerated biological aging. Even though some of the effect may be attributable to baseline differences in health behaviors and other individual differences, or the way those factors influence further selection into neighborhoods, there is a continuing significant effect of neighborhood characteristics on accelerated aging even after taking into account potential bias due to selection effects.

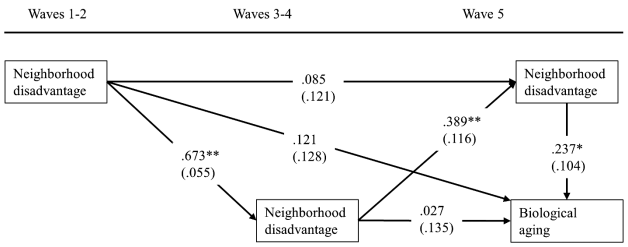

Figure 3. Mediated model of the relationship between early and later neighborhood disadvantage on biological aging. The values presented are standardized parameter estimates. $^{**}p \le .01$; $^{*}p \le .05$ (two-tailed tests).

Table 2. Effects of Cumulative Exposure to Neighborhood Disadvantage on Biological Aging

	b	*SE*	95% CI	*p* value
Unadjusted pooled regression				
Model 1: without control variables	1.002	0.355	0.296, 1.707	.006
Model 2: with control variables	0.671	0.226	0.223, 1.119	.004
Marginal structural model (weighted by stabilized IPTW)				
Model 3: without time-invariant variables	0.903	0.348	0.213, 1.594	.011
Model 4: with time-invariant variables	0.752	0.241	0.272, 1.232	.002

Note: Coefficients and robust standard error are obtained from a pooled regression using five waves of data. $^{\dagger}p \le .10$, $^{*}p \le .05$, $^{**}p \le .01$ (two-tailed tests).

Discussion

Although the link between neighborhood context and healthy aging has long-attracted attention in the social science and public health literatures, this research has been plagued by methodological questions. Studies have often relied on self-report measures of aging and fail to control potential neighborhood selection effects. Thus, past research on the effect of neighborhood context on aging is vulnerable to concerns that effect estimates may have been overestimated or worse, entirely spurious. To overcome these limitations, the current study used newly developed epigenetic methods to directly assess rate of biological aging and utilized marginal structural models to control for selection effects. This approach entailed a more stringent test of the causal effect of neighborhood disadvantage on biological aging than has been provided in past studies.

Across several analytic strategies, our findings provide consistent evidence that residing in a disadvantaged neighborhood accelerates biological aging. This effect remained after including stabilized IPTW in our regression models (Robins, 2000) to control for neighborhood selection effects. Specifically, our results indicated that, even after controlling potential effects of health behavior and other individual differences, a one standard deviation increase in neighborhood disadvantage increased an individual's methylomic age by roughly 9 months. More broadly, this finding supports the theoretical view that the ambient stress of disadvantaged neighborhoods fosters biological wear and tear throughout the life course or what has been labeled biological weathering (Geronimus, 2010, 2013), and that effects are not entirely attributable to individual level financial strain or health behaviors engendered by neighborhood context.

The current study is not without limitations. First, because our sample was limited to middle-aged African American women it does not allow us to test for gender differences. It might be that the effects would be different for African American men. In some respects, however, this limitation might be seen as a strength. Indeed, there is evidence that mortality rates for Black women worsened after 1990, and that the most prominent differences in health between Black and White women are in middle age (Geronimus et al., 2010). Numerous studies have also indicated that Black women are disproportionally likely to reside in extremely disadvantaged neighborhoods (Peterson & Krivo, 2010), making them an ideal sample for investigating the link between neighborhood conditions and aging. A second limitation is that the current sample did not allow us to test for differences across ethnic or racial groups. According to the racial invariance hypothesis (Sampson, 2012), neighborhood conditions equally affect people of all racial/ethnic groups. Still, the current results clearly need to be replicated with samples that are more ethnically diverse and that include males.

Third, our results may have been influenced by unmeasured confounds. We included a comprehensive list of potential confounders that have been previously reported to be associated with neighborhood disadvantage (Sampson, Sharkey, & Raudenbush, 2008) and biological aging (Simons et al., 2016). However, as with all observational studies, unmeasured and residual confounds may remain that potentially influence the effect sizes. Fourth, the current study was limited by its small sample size. Although the findings clearly need to be replicated with a larger sample to increase confidence in generalizability of the findings, our study had enough statistical power to test our hypotheses. Fifth, our study assessed methylation status at only one point in time. Future studies should focus on longitudinal changes in DNA methylation. Unfortunately, it is difficult to generate such data sets given the challenge of drawing blood from subjects scattered across neighborhoods and the costs of methylation assays. Finally, our assessment of methylation relied on peripheral whole blood. Some methylation patterns are tissue specific and vary depending on cell types, indeed this is a primary mechanism for cell-type differentiation. Thus, we measured methylomic age through the Hannum and colleagues (2013) measure which is designed to be used with peripheral blood samples. To control for cell type-specific effects, we also estimate our models by controlling for individual differences in mononuclear white blood cell types. That said, it would be interesting to examine the effect of neighborhood conditions on methylomic age using tissue samples other than blood.

In conclusion, recent studies have reported that methylomic aging is a robust predictor of mortality and chronic diseases. Marioni and colleagues (2015), for example, used four longitudinal data sets and found that, even after controlling for socioeconomic status and various health-related behaviors, individuals had a 21% greater mortality risk when their biological age was five years greater than their chronological age. In another study, Zheng and colleagues (2016) revealed that, even after controlling for telomere length, individuals with a biological age one year greater than their chronological age showed a 6% increased risk of getting cancer within three years and a 17% increased risk of cancer death within five years. Therefore, new indices of epigenetic aging provide a more direct method of assessing biological aging than what has been available. The current research is the first to demonstrate a robust link between neighborhood disadvantage and epigenetic aging that holds even after adjusting for selection issues. This finding points to the importance of intervention strategies based upon neighborhood renewal and regeneration as avenues for reducing the personal and societal costs of chronic illness due to accelerated aging.

Supplementary Material

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

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Conflict of Interest

The authors declare no conflicts of interest.

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