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### **Examining the Cross-sectional Association Between Neighborhood Conditions, Discrimination, and Telomere Length in a Predominantly African American Sample**

**Wendy M. Troxel**1, **Jaime Madrigano**2,3, **Ann C. Haas**1, **Tamara Dubowitz**1, **Andrea L. Rosso**4, **Aric A. Prather**5, **Madhumita Ghosh-Dastidar**6, **Andrea M. Weinstein**7, **Meryl A. Butters**7, **Albert Presto**8, **Tiffany L. Gary-Webb**<sup>4</sup>

<sup>1</sup>Social and Economic Well-Being Division, RAND Corporation, Pittsburgh, PA 15213, USA

<sup>2</sup>Social and Economic Well-Being Division, RAND Corporation, Arlington, VA, USA

<sup>3</sup>Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

<sup>4</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

<sup>5</sup>Department of Psychiatry and Behavioral Sciences, School of Medicine, University of California San Francisco, San Francisco, CA, USA

<sup>6</sup>Economics, Sociology, and Statistics Division, RAND Corporation, Santa Monica, CA, USA

<sup>7</sup>Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

<sup>8</sup>Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA

#### **Abstract**

Disproportionate exposure to adverse neighborhood conditions and greater discrimination may contribute to health disparities among African Americans (AAs). We examined whether adverse neighborhood conditions, alone or in conjunction with discrimination, associate with shorter leukocyte telomere length among a predominantly AA cohort. The sample included 200 residents from two low-income neighborhoods (96% AA; mean age = 67 years). Perceived neighborhood conditions and discrimination were surveyed in 2018, and objective neighborhood conditions (total crime rate, neighborhood walkability, ambient air pollution  $(PM<sub>2.5</sub>$ , black carbon)) were collected in 2017/2018. Relative telomere length (T/S; ratio of telomeric DNA to a single-gene

<sup>✉</sup>Wendy M. Troxel, wtroxel@rand.org.

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**Competing Interests** The authors declare no competing interests.

**Ethics Approval** All aspects of this study have been approved by the RAND Human Subjects Protection Committee and the University of Pittsburgh Institutional Review Board, and participants within this study have been treated in accordance with the American Psychological Association's ethical standards.

**Consent to Participate** Written informed consent was obtained from all study participants.

**Consent for Publication** No individual-level data or images are included. Informed consent was provided for the publication of data in aggregate form, with no personal identifiers included.

copy) was assessed from blood samples. Linear regression models estimated the main effects of each neighborhood condition and discrimination and their interactions on the T/S ratio. Less walkable neighborhoods were associated with shorter telomeres. Higher air pollution ( $PM<sub>2.5</sub>$ ) was associated with shorter telomeres among those experiencing greater discrimination. Findings highlight the importance of understanding the intersecting influences of historic and contemporary sources of systemic racism and how they contribute to accelerated aging among adults.

#### **Keywords**

Neighborhood disadvantage; Discrimination; Telomere length; Socioeconomic status; African American; Health disparities

> The legacy of structural racism, including discriminatory land use and housing policies, has led to racially segregated and disinvested neighborhoods that exist today across the USA [1, 2]. African American (AA) individuals are more likely to live in neighborhoods characterized by socioeconomic disadvantage, greater exposure to crime and air pollutants, and lower access to health-promoting resources [2–5]. In turn, living in a disadvantaged neighborhood is associated with an increased risk of morbidity and mortality, even after adjusting for individual socioeconomic status (SES) [6–9].

However, only a handful of studies have investigated potential cellular mechanisms underlying associations between neighborhood disadvantage and health. Leukocyte telomere length is a potential biomarker of cellular aging, related both to indicators of chronic stress and to increased risk of morbidity and mortality [10]. Shortening of telomeres (via loss of base pairs) occurs progressively across the lifespan and is considered a natural part of aging. However, social and environmental stressors can trigger premature telomere shortening [11].

Repeated exposure to detrimental social and environmental conditions may contribute to accelerated telomere shortening via direct and indirect mechanisms [11]. For example, low-income AA urban populations are disproportionately exposed to poor air quality [12, 13] as well as inferior features of the built environment such as reduced access to greenspace or easily walkable neighborhoods and reduced access to healthy food options [3]. Such environmental exposures can directly affect telomeres via oxidative stress and inflammatory pathways [14]. Furthermore, these exposures are often coupled with adverse social conditions stemming from economic deprivation and racial injustice, including stress related to living in unsafe neighborhoods and exposure to interpersonal discrimination. In turn, these aspects of the social environment may contribute to telomere shortening via stressrelated physiological pathways, including inflammation, neuroendocrine dysregulation, and autonomic hyperarousal.

Only a handful of studies have investigated multiple aspects of neighborhood conditions in association with TL, with even fewer studies including AA individuals [15–17]. The extant results are equivocal, in part due to differences in sample composition and measurement of neighborhood characteristics and TL. For example, several studies have demonstrated associations between particulate matter air pollution and shortened TL, although much of this work has been conducted within specific populations occupationally exposed to

environmental hazards [18]. In terms of broader neighborhood conditions, Massey et al. [19] found that a neighborhood socioeconomic index comprising several census-tract indicators (e.g., education, poverty rate, unemployment) was associated with shorter TL. In contrast, several studies have found significant associations between perceived neighborhood conditions and shorter telomeres, but not neighborhood socioeconomic disadvantage [20, 21]. These findings are consistent with the premise that activation of the stress response is driven by the individuals' perception of the conditions [22].

Importantly, perceptions of neighborhood conditions and the resultant impact on stress physiology and cellular aging are shaped by a confluence of intersecting influences and exposures throughout the individual's lifespan. With few exceptions 16, 23, 24], there has been a scant investigation of how other contextual factors may influence the association between perceptions of neighborhood conditions and TL.

Exposure to interpersonal discrimination is an important stressor that may interact with neighborhood conditions to influence TL. Several studies have investigated the association between discrimination and TL, with mixed results [10, 25]. Neighborhood conditions may buffer or exacerbate the impact of exposure to discrimination. For example, feeling safe in one's neighborhood could buffer the stress-related negative impact of discrimination on TL. Alternatively, adverse neighborhood conditions may potentiate the negative effects of discrimination on TL. To our knowledge, only one prior study [24] has investigated potential interactive effects, finding a greater shortening of telomeres over a 10-year followup period among those who lived in less socially cohesive neighborhoods and who had been exposed to multiple domains of discrimination (e.g., housing, workforce), compared to those who had experienced no discrimination. Understanding interactions between neighborhoodlevel conditions and individual exposure to discrimination is critical to identify potential mitigating factors that could be targets of multi-level interventions, including policy change.

The current study aims to extend the existing research on neighborhood conditions and TL in a sample of mid-life to older, predominantly AA adults, living in two low-income urban neighborhoods. We examine the association between (1) perceived neighborhood social conditions, including safety and cohesion, and (2) objective measures of neighborhood conditions, including crime rates, walkability, and air pollutants, with TL. Next, we examine how both perceived and objectively measured neighborhood conditions independently, and in interaction with discrimination, associate with TL. We hypothesize that poorer neighborhood conditions including lower levels of perceived safety and cohesion, less walkable neighborhoods, higher levels of crime, and higher levels of  $PM_{2.5}$  and black carbon (BC) exposure will be associated with shorter TL. We also predict that greater exposure to interpersonal discrimination will be associated with shorter TL and will interact with neighborhood conditions, such that more positive neighborhood conditions will buffer the negative impact of discrimination on telomeres.

#### **Methods**

#### **Study Sample**

Data comes from an ancillary study (THINK PHRESH) of the Pittsburgh Hill/Homewood Research on Neighborhood Change and Health (PHRESH). As described in detail previously, the original PHRESH cohort includes a random sample of households from two low-income, predominantly AA neighborhoods (Hill District, Homewood) in Pittsburgh, Pennsylvania [3]. Data collection began in 2011, with subsequent data collection occurring in 2013, 2014, 2016, 2017 (street segment audits only), and 2018. In 2018, with supplemental funding, a subsample of PHRESH participants were invited and agreed to participate in the THINK PHRESH ancillary study ( $N = 256$ ), which added assessments of cognitive function and impairment to the existing PHRESH assessments. To be eligible for THINK PHRESH, participants had to be 50 years or older, have had at least one prior wave of PHRESH data, and be able to complete the cognitive assessments. For the current analyses, participants also had to have stored blood from a previous PHRESH assessment and to provide consent for allowing their stored blood to be assayed for TL, resulting in an analytic sample of 200 for the present analyses. All aspects of this study have been approved by the RAND Human Subjects Protection Committee and the University of Pittsburgh Institutional Review Board, and participants within this study have been treated in accordance with the American Psychological Association's ethical standards.

#### **Measures**

**Perceived Neighborhood Conditions—Social cohesion was measured with a** validated, five-item Likert scale, ranging from 1 (strongly agree) to 5 (strongly disagree) [26]. Participants indicated how strongly they agree or disagree with various statements (e.g., "People around here are willing to help their neighbors";  $\alpha = 0.84$ ) [26]. Scores were coded such that higher scores indicate greater social cohesion.

Perceived neighborhood safety was assessed by averaging four items, each rated on a 5-point Likert scale, ranging from 1 (strongly agree) to 5 (strongly disagree). Participants indicated how strongly they agree or disagree with various statements (e.g., "You feel safe walking in your neighborhood during the day";  $\alpha = 0.71$ ). Scores were coded such that higher scores indicate greater perceived safety [27].

**Objective Neighborhood-level Conditions—The walkability index [28] was derived** from neighborhood street segment audits which were collected through direct observation by trained data collectors in both neighborhoods in 2017. The walkability index included items on traffic signs, pedestrian crossings, sidewalks, lighting, transit, and mixed residential and commercial use. Items were summed and averaged across each street segment ( $\alpha$  = 0.77) [28]. The walkability index ranges from 0 to 22, with higher scores indicating greater walkability.

Neighborhood crime was calculated using incident-level total crime data provided by the City of Pittsburgh police department and ArcGIS 10.2 software. We calculated street network distances from each household to each approximate crime location. We were able

to geocode 95% of the incidents using the address information from the raw data. For each household, we summed the total number of 2018 crimes that occurred within a 1-km network distance to arrive at the household buffer for total crimes in 2018.

Annual ambient air pollutant exposures were estimated for 2018 using previously published land use regression (LUR) models for P  $M<sub>2.5</sub>$  and BC [29, 30]. These two pollutants were selected based on prior work that demonstrated the association between P  $M<sub>2.5</sub>$ , BC, and TL, as well as other aging-related conditions [18, 31, 32]. The LUR models were trained on mobile sampling data collected at sites across Allegheny County, including in the target neighborhoods, in 2013–2014 [30]. The models were built using forward variable selection following the ESCAPE [33] protocol with tenfold cross-validation. LUR models are resolved at a nominal 10-m resolution, and exposure estimates were assigned to participants based on the residential address. Given the temporal mismatch between air pollutant data collection (2013–2014) and other data (2018), we performed a temporal adjustment to the air pollutant surfaces predicted by the LUR models. We multiplied LUR model outputs in the base year of 2013 by a temporal factor using Allegheny County Health Department (ACHD) air quality monitoring data for  $PM<sub>2.5</sub>$  and elemental carbon (EC, a proxy for BC). The temporal factor was calculated as the (annual mean concentration of  $PM_{2.5}$  or EC at the ACHD site in 2018)/(annual mean concentration of  $PM_{2.5}$  or EC at the ACHD site in the base year of 2013). The ACHD site is representative of typical urban air pollutant conditions in Pittsburgh, located approximately 2 km from the Hill District and 5 km from Homewood.

**Interpersonal Discrimination—**Exposure to interpersonal discrimination was measured using the Everyday Discrimination Scale, Short Version [34]. Participants were asked how often they had each of five experiences in their day-to-day life (e.g., "Being treated with less courtesy or respect than other people"), with response options for each item ranging from 0 (never) to 5 (almost every day). Scores reflect the sum of responses, ranging from 0 to 25, with higher scores indicating more frequent experience of discrimination. The internal reliability of this scale was adequate ( $\alpha = 0.81$ ).

**Telomere Length Measurement—**Total genomic DNA from whole blood samples collected via venipuncture in 2016 and/or in 2018 and stored at − 80° were used to measure TL. The year of blood data collection was not statistically significantly associated with telomere length or with any neighborhood characteristics.

DNA was extracted from whole blood stored at − 80 °C with the QIAamp DNA blood mini kit (QIAGEN cat# 51,106). DNA quantity and quality were assessed by OD260/OD280 and OD260/OD230 on a NanoDrop 2000C spectrophotometer. The quality control criteria are concentration greater than 10 ng/ul, OD260/OD280 between 1.7 and 2.0 and OD260/OD230 greater than 1.0. If the DNA did not meet the QC, another aliquot of whole blood was used to repeat the DNA extraction. DNA was stored at − 80 °C for approximately 3 months and experienced up to 3 freeze–thaw cycles; DNA integrity was not assessed.

The telomere length measurement assay is adapted from the originally published method by Cawthon [35]. The telomere thermal cycling profile (T-PCR) consists of denature at 96 °C

for 1 min; denature at 96 °C for 1 s, anneal/extend at 54 °C for 60 s, with fluorescence data collection, 30 cycles. Cycling for the single-copy gene (S-PCR) consists of denature at 96 °C for 1 min; denature at 95 °C for 15 s, anneal at 58 °C for 1 s, extend at 72 °C for 20 s, 8 cycles; followed by denature at 96 °C for 1 s, anneal at 58 °C for 1 s, extend at 72 °C for 20 s, hold at 83 °C for 5 s with data collection, 35 cycles.

The telomere qPCR primers were tel1b [5'-CGGTTT(GTTTGG)5GTT-3'], used at a final concentration of 100 nM, and tel2b [5'-GGCTTG(CCTTAC)5CCT-3'], used at a final concentration of 900 nM. The single-copy gene (human beta-globin) qPCR primers were hbg1 [5'-GCTTCTGACACAACTGTGTTCACTAGC-3'], used at a final concentration of 300 nM, and hbg2 [5'-CACCAACTTCATCCACGTTCACC-3'], used at a final concentration of 700 nM. The final reaction mix consisted of the following: 20 mM Tris-hydrochloride, pH 8.4; 50 mM potassium chloride; 200 μM each deoxyribonucleotide triphosphate; 1% dimethyl sulfoxide; 0.4 × SYBR green I; 22 ng Escherichia coli DNA; 0.4 units of platinum Taq DNA polymerase (Invitrogen Inc., Carlsbad, CA), and approximately 6 ng genomic DNA per 11 μl reaction. A threefold serial dilution of a commercial human genomic DNA (Sigma cat # 11,691,112,001) containing 26 ng, 8.75 ng, 2.9 ng, 0.97 ng, 0.324 ng, and 0.108 ng of DNA was included in each PCR run as the reference standard. The quantity of targeted templates in each sample was determined relative to the reference DNA sample by the maximum second derivative method in the Roche LC480 program. The reaction was carried out in a Roche LightCycler 480 in 384-well plates, with triplicate wells for each sample. Dixon Q test was used to exclude outliners from the triplicates. The average of the T and S triplicate wells after outliner removal was used to calculate the T/S ratio for each sample. The same reference DNA was used for all PCR runs. The PCR efficiencies of single-copy gene and telomere primers are  $88.1 \pm 2.4\%$  and  $85.6 \pm 3.4\%$ , respectively.

A set of 8 DNA samples from human cancer cell lines were used as controls to adjust batch effects. Additionally, a subset of samples from each batch (up to 96 samples) were run together in the same run to adjust the batch-to-batch variability. The T/S ratio for each sample is measured twice. If the duplicate T/S value and initial value vary by more than 7%, the sample is run a third time and the two closest values will be reported. The inter-assay coefficient of variation in this study was  $2.1 \pm 1.4\%$ .

**Covariates—**The following sociodemographic characteristics are potential confounders of associations between neighborhood conditions and TL and were included in the models as covariates: age, gender (male or female), years of education, marital status (married or living with a partner versus living alone), presence of children in the household, annual household income, neighborhood of residence (Hill District or Homewood), and length of neighborhood residence. Race/ethnicity was not included as a covariate because 96% of the sample identified as AA or Black. Follow-up models additionally controlled for health behaviors and conditions that have previously demonstrated associations with TL and may, at least in part, explain associations between neighborhood conditions and TL, including current smoking status (current smoker versus non-smoker), body mass index (BMI; from interviewer-measured height and weight), hypertension status (measured blood pressure 140/90 mm Hg, taking hypertensive medication, or reported diagnosis of hypertension),

and high blood sugar status (measured glycosylated hemoglobin 6.5%, taking diabetes medication, or reported diagnosis of diabetes).

#### **Statistical Analysis**

Due to the specific eligibility criteria for enrollment in THINK PHRESH, we first examined whether participants with telomere data (the analytic sample) differed from participants ages 50 years or older in the 2018 PHRESH data collection. Given several differences in observed demographic characteristics (i.e., age, education, and sex), we used survey weighting so that the analytic sample represented the eligible sample PHRESH participants in 2018. Survey weights were used for both descriptive statistics and modeling. Next, descriptive characteristics for study neighborhood conditions, discrimination, covariates, and T/S ratio were calculated overall and by neighborhood (Hill District versus Homewood). Primary analyses involved linear regression models to examine the main effects of each neighborhood condition and discrimination (entered in separate models) on the T/S ratio, controlling for only sociodemographic characteristics (model 1), or all covariates including health behaviors and conditions (model 2). The final model (model 3) added an interaction term between discrimination and each neighborhood condition (separately). Neighborhood conditions and discrimination were converted to z-scores. Thus, regression coefficients can be interpreted as the covariate-adjusted association between a 1 standard deviation (SD) increase in the predictor and a change in the T/S ratio.

To aid in the interpretation of significant results, we estimated the equivalent association between age and the T/S ratio. Neighborhood condition and discrimination variables were missing for a small number of cases (between 1 and 6 missing in the analytic sample of 200). Therefore, sample sizes range between 194 and 199 cases. Sensitivity analyses estimated all regression models subset to only AA participants ( $n = 192$ ). However, results were similar when restricting the sample to African Americans. Thus, we report full sample results only. We also implemented an additional set of sensitivity analyses that adjusted for the year of blood collection (2016 or 2018). Results were very similar to those reported herein. Therefore, we present results only with a priori determined covariates. Analyses were conducted using SAS software, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

#### **Results**

Table 1 presents descriptive statistics. Participants are primarily AA women with a mean age of 67 years  $(SD = 9)$  and generally of low SES status with a high number of health conditions endorsed by the participant.

Table 2 shows the results of the regression models including the main effects with base set of covariates (model 1) and all covariates (model 2). In model 1, the walkability index was positively associated with TL, such that a one-SD increase in the walkability index was associated with a 0.031 higher average T/S ratio (95% CI = [0.011, 0.052],  $p < 0.01$ ). To facilitate interpretation of the magnitude of associations, a 1-year increase in age was associated with a 0.008 lower average T/S ratio (95% CI = [0.006, 0.010],  $p < 0.001$ ). Using this result for age, we estimate that for each 1 SD decrease in the walkability index,

There was one significant interaction observed between air pollution and discrimination. Specifically, there was a stronger inverse association between P  $M_2$ , and TL among those with higher levels of discrimination (β = -0.016, 95% CI =  $[-0.032, < -0.001]$ ,  $p < 0.05$ ) adjusting for all covariates. No other interaction terms were significant (analyses not shown).

#### **Discussion**

The current study extends the small, extant literature on neighborhood conditions and TL by examining the association between multiple neighborhood conditions, including air pollution, and TL in a sample of predominantly AA residents living in two racially segregated neighborhoods in the city of Pittsburgh. Furthermore, recognizing the intersection of both systemic and interpersonal racism, we examine how interpersonal discrimination, alone or in conjunction with neighborhood conditions, associates with TL in this sample.

Findings demonstrate a statistically significant association between neighborhood walkability and TL, such that less walkable neighborhoods are associated with shorter telomeres, even after adjustment for sociodemographics, health behaviors, and conditions, including smoking and cardiometabolic risk factors. The magnitude of this association was roughly equivalent to that of the effect of aging by 3.9 years on telomeres. More walkable neighborhoods may benefit cellular aging by increasing physical activity and reducing sedentary activity [36]. Future longitudinal research is needed to examine the role of physical activity as a possible mediator of associations between neighborhood walkability and telomere length as well as the potential impact of policy-level interventions on neighborhood conditions on telomere shortening.

We did not find significant associations between social environment conditions, including neighborhood safety, cohesion, or crime and TL. These findings are in contrast with the limited prior studies, utilizing national samples that have found significant associations between a variety of neighborhood conditions and TL [17]. Similarly, Geronimus and colleagues found significant associations between greater neighborhood satisfaction as well as (counterintuitively) lower neighborhood safety and longer TL among AA individuals living in Detroit. The limited and somewhat mixed literature on social environmental characteristics and TL suggests that geographic and age differences across the sample may contribute to divergent findings [16].

Although we did not find significant main effects of air pollutants on TL, we did find evidence for a significant interaction between  $PM_{2.5}$  and discrimination on TL. Among individuals experiencing higher levels of discrimination, there was a significant association between greater  $PM_{2.5}$  exposure and shorter TL. This finding is consistent with the growing body of literature that demonstrates the synergistic effects of social stressors and environmental hazards on population health [37, 38]. For example, prior literature has found a 1.5 times greater prevalence of diabetes among those with both a high number of adverse childhood experiences and higher exposure to particulate air pollution compared to those

with a lower number of adverse childhood experiences and lower particulate air pollution exposure [39]. The present study adds to the literature by providing further evidence for the role of epigenetic mechanisms in the interactive effects of social stressors and air pollution.

Consistent with prior research [16], we did not find a significant main effect of discrimination on TL. The lack of a significant main effect of discrimination may reflect that this study took place in two racially segregated neighborhoods, which may reduce exposure to interpersonal discrimination.

Study results must be interpreted within the context of several methodological limitations. First, the cross-sectional design precludes inferences regarding causality. Second, the relatively small sample comprised primarily of low-income AA adults and from a constrained geographic area may limit the generalizability of the results. However, this is a population that is underrepresented in biological aging research and faces a disproportionate burden in terms of chronic disease and premature mortality [8]. Finally, we did not measure all potential neighborhood characteristics that may associate with TL, nor did we assess whether TL serves as a biological mechanism linking neighborhood conditions with clinically relevant disease endpoints.

#### **Conclusions**

Despite these limitations, our study makes several contributions to the nascent literature on neighborhood conditions and TL. Specifically, this study contributes to our understanding of the interactive effects between social stressors (i.e., discrimination) and environmental hazards seen across a wide variety of health outcomes, which is critical in order to inform multi-level intervention efforts. Furthermore, the focus of our study is on a predominantly African American cohort of adults living in racially segregated neighborhoods, which is an underrepresented population exposed to both historic and contemporary sources of systemic racism and interpersonal discrimination. Therefore, findings are critical to inform novel intervention efforts that target both upstream and downstream causes of health disparities.

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De-identified data is available by request.

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 $^4$ Individuals are coded as having hypertension if they had high measured hypertension (  $\,$  90 mmHg for diastolic blood pressure or  $\,$  140 mmHg for systolic blood pressure), reported currently taking medication for h Individuals are coded as having hypertension if they had high measured hypertension (≥ 90 mmHg for diastolic blood pressure or ≥ 140 mmHg for systolic blood pressure), reported currently taking

medication for high blood pressure, or reported a prior diagnosis of hypertension

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**Table 1**

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diagnosis of diabetes

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 $b$ <sub>ndividuals</sub> are coded as having high blood sugar if they had high measured blood sugar (hemoglobin A1c 6.5%), reported currently taking medication for diabetes or high blood sugar, or reported a prior diagnosis of dia Individuals are coded as having high blood sugar if they had high measured blood sugar (hemoglobin A1c ≥ 6.5%), reported currently taking medication for diabetes or high blood sugar, or reported a prior

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# **Table 2**

Results of crosssectional linear regression models predicting telomere length (T/S ratio) from discrimination and neighborhood factors Results of crosssectional linear regression models predicting telomere length (T/S ratio) from discrimination and neighborhood factors



child in the home. Model 2 additionally adjusts for the following health behavior and conditions covariates: body mass index, current smoking status, hypertension, and high blood sugar. Sample sizes range child in the home. Model 2 additionally adjusts for the following health behavior and conditions covariates: body mass index, current smoking status, hypertension, and high blood sugar. Sample sizes range deviation = 1 before including as predictors in regression models. Model 1 adjusts for the following covariates: neighborhood, age, gender, years in the neighborhood, income, education, marital status, and deviation = 1 before including as predictors in regression models. Model 1 adjusts for the following covariates: neighborhood, age, gender, years in the neighborhood, income, education, marital status, and Each of the discrimination and neighborhood factor variables is entered into separate models. Discrimination and neighborhood factor variables were converted to z scores to have mean = 0 and standard Each of the discrimination and neighborhood factor variables is entered into separate models. Discrimination and neighborhood factor variables were converted to z-scores to have mean = 0 and standard by the outcome from 194 to 199. B (95% CI) by the outcome from 194 to 199. B (95% CI)

 $p < 0.05$ 

\*\*  $p < 0.01$  \*\*\*  $p < 0.001$