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Cumulative risk exposure and child cellular aging in a Dutch low-risk community sample

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Abstract

One of the proposed mechanisms linking childhood stressor exposure to negative mental and physical health outcomes in later life is cellular aging. In this prospective, longitudinal, and pre-registered study, we examined the association between a cumulative pattern of childhood risk exposure from age 6 to age 10 (i.e., poor maternal mental health, parental relationship problems, family/friend death, bullying victimization, poor quality friendships) and change in two biomarkers of cellular aging (i.e., telomere length, epigenetic age) from age 6 to age 10 in a Dutch low-risk community sample ($n = 193$). We further examined the moderating effect of cortisol reactivity at age 6. Ordinary Least Squares regression analyses revealed no significant main effects of childhood risk exposure on change in cellular aging, nor a moderation effect of child cortisol reactivity. Secondary findings showed a positive correlation between telomere length and cortisol reactivity at age 6, warranting further investigation. More research in similar communities is needed before drawing strong conclusions based on the null results.

Roseriet Beijers and Ilse ten Thije contributed to the work equally.

1 | INTRODUCTION

1.1 | Childhood stress exposure and cellular aging

Exposure to stress during childhood is associated with poor mental health (Pirkola et al., 2005) and physical health (Repetti et al., 2002), including cardiovascular disease (Pierce et al., 2020) and cancer (Kelly-Irving et al., 2013). Such results raise mechanistic questions about *how* childhood stress exposure gets under the skin and affects health later in life. One proposed mechanism is accelerated cellular aging (Shalev, 2012). In the preregistered study reported herein, we examine the association between cumulative childhood stressor exposure (i.e., poor maternal mental health, parental relationship problems, family/friend death, bullying victimization, poor quality friendships) and change in two biomarkers of cellular aging (i.e., telomere length, epigenetic age), as well as the possible moderating effect of cortisol reactivity, in a Dutch low-risk community sample.

1.2 | Cellular aging

One form of cellular aging involves the gradual loss of the cell's regenerative capacity, eventually resulting in cellular senescence, defined as the termination of cell division (Campisi & d'Adda di Fagagna, 2007). Telomeres are one chromosomal feature that has been used to assess biological age. Telomeres are protective regions composed of TTAGGG nucleoprotein repeats at the end of all chromosomes. With each cell division telomeres erode until the Hayflick limit is reached, and the cell enters a state of senescence (Shalev, Entringer, et al., 2013). In adulthood, shorter telomeres are associated with multiple diseases such as coronary heart disease (for a review see Haycock et al., 2014), several types of cancer (for reviews see Ma et al., 2011 and Wentzensen et al., 2011), and Alzheimer's disease (for a review see Forero et al., 2016). Just as notable, evidence indicates that telomeres erode more rapidly in individuals exposed to greater stress—of a variety of kinds—in childhood (Pepper et al., 2018; Ridout et al., 2018). Shorter telomeres are linked to the experience of maltreatment (Asok et al., 2013; Ridout et al., 2019), childhood violence (Drury et al., 2014; Shalev, Moffitt, et al., 2013), growing up in low SES families (Needham et al., 2012) and severely deprived institutions (Drury et al., 2012; Humphreys et al., 2016). Important to appreciate is that virtually all such work has been carried out in high-adversity risk (community) samples, thus raising questions about whether similar accelerating effects of perhaps less extreme forms of stress prove to be detectable in low-adversity risk community samples.

A second form of cellular aging involves changes in DNA methylation at specific sites across the genome (usually CpG dinucleotides) (Horvath & Raj, 2018) with estimates of epigenetic age correlated with chronological age (Horvath, 2013). Notably, epigenetic age acceleration, defined as increased epigenetic age relative to chronological age has been associated with greater all-cause mortality in cohorts of older adults (Chen et al., 2016). Just as notable, once again, is that accelerated epigenetic aging is also associated with childhood stressor exposures, including neighborhood violence (Jovanovic et al., 2017), general adversity (Marini et al., 2020) and cumulative threat—but not deprivation-related adversity (Sumner et al., 2019).

Important to note is that the results just summarized are based on the Horvath "epigenetic clock." Recent evidence indicates that this index is less accurate in pediatric samples (McEwen et al., 2019), possibly due to the relatively rapid change in DNA methylation in childhood as compared to adulthood (Alisch et al., 2012). The current study is the first to investigate effects of childhood stressor exposure on epigenetic aging using the newly developed Pediatric-Buccal-Epigenetic (PedBE) clock, a measure found to be highly predictive of epigenetic age in pediatric buccal cells (McEwen et al., 2019). A recent study has shown that epigenetic age as measured with the PedBe-clock is affected by prenatal adversity (McGill et al., 2022), but effects of childhood stress are yet to be tested.

1.3 | Differential susceptibility to effects of stress

Ever more evidence makes clear that children vary in their susceptibility to effects of a variety of developmental experiences and exposures. According to the classic diathesis-stress model (Monroe & Simons, 1991), some individuals are presumed—and have been found—to be more vulnerable to the negative effects of adversity experienced across the life course. A more recent, alternative model known as "differential susceptibility" stipulates that some individuals are more susceptible to both positive and negative environmental exposures, making them not so much vulnerable as more generally developmentally plastic (Belsky, 1997, 2005; Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Ellis et al., 2011).

When it comes to person characteristics thought to function as plasticity factors, the just-cited work calls attention to genetic, temperamental, and physiological factors. With regard to physiological factors, the biological-sensitivity-to-context theorizing of Boyce and Ellis (2005) proposes that children, but perhaps not adolescents or adults, who are more physiologically reactive prove to be more susceptible to effects of both supportive

and unsupportive environmental conditions (i.e., "for better and for worse", Belsky et al., 2007). One such plasticity index focuses on cortisol, with cortisol reactivity reflecting the amount of cortisol that is released when an individual is in a stressful situation. In the context of stress and biological aging, cortisol reactivity has not yet been investigated as susceptibility factor.

1.4 | Current study

Herein, we test the proposition that greater childhood stress between 6 and 10 years of age will predict shorter telomeres and/or older epigenetic age at age 10, corrected for telomere length/epigenetic age at age 6, when cellular age was first measured. Next, we also test the proposition that cortisol reactivity will moderate such main effects. More specifically, we predict that more physiologically reactive children will age more quickly if subjected to high levels of stress, as compared to children with less reactive profiles. In order to measure childhood stressor exposure, we follow the commonly used method of creating a cumulative risk score (e.g., Drury et al., 2014; Shalev, Moffitt, et al., 2013; Sumner et al., 2019). This cumulative risk score includes the following childhood stressors: parental relationship problems, maternal mental health problems, death of someone close to the family, bullying victimization, and poor quality of friendships. These five risk variables are chosen based on prior literature (Kiecolt-Glaser et al., 2011; Shalev, Moffitt, et al., 2013) and the availability of their measurement in the interval between the two measurements of cellular aging. Unlike many of the prior studies cited, we rely on prospective data rather than retrospectively reported information on childhood adversity—the latter being more prone to recall biases (Hardt & Rutter, 2004). Additionally, we focus on a low- rather than high-risk community sample, again making this study quite different than many related ones in the literature.

2 | METHOD

2.1 | Participants

This study was preregistered as "The main and moderated effect of risk exposure on child cellular aging" (#65265) at AsPredicted (https://aspredicted.org/j7pu6.pdf). The data used in this study came from a longitudinal project on psychobiological development in children (Basal Influences on Child Development [BIBO] project; see also Beijers et al., 2011). Pregnant women were recruited through midwife practices in and around Nijmegen, The

Netherlands. Inclusion criteria for the BIBO study were as follows: uncomplicated singleton pregnancy with term delivery, no drug use during pregnancy, no major maternal physical and/or mental health problems during pregnancy, a 5-min infant Apgar score of ≥7, an infant birth weight ≥2500 g, and a clear understanding of the Dutch language. This resulted in a sample of 193 mothers and their infants (for demographics see Table 1). Ethical approval was obtained from the Social Science Ethical Committee of the Radboud University, following the Helsinki Declaration (#ECG300107). All mothers provided written informed consent.

Of the 193 parent–child dyads that were originally included in the BIBO study, 160 parent–child dyads were still participating at child age 10. Participants with missingness on the outcome variables (i.e., age-corrected telomere length or epigenetic aging at age 10) were excluded from further analyses. This left us with two samples: one for the analyses with telomeres as outcome ($n = 156$), and one for the analyses with epigenetic age as outcome ($n = 158$). Excluded participants did not differ significantly from the other participants in terms of sex, maternal education, BMI, cumulative risk scores, telomere length at age 6, epigenetic age at age 6, and cortisol reactivity. An ad hoc power analysis was performed to determine whether a sample size of 158 would allow us to detect the effect when present in this sample. This power analysis indicated that a small to medium effect size (Cohen's $f^2 = 0.05$) is detectable in this sample size with a power of 80% and an alpha of .05.

2.2 | Procedure

At child age 6, a researcher visited the child at school with a mobile lab. In 8 cases, children were visited at home. The visits took place in the afternoon of a regular school day between 12.15 and 15.15 h. As research indicates that recent food intake and physical activity can affect cortisol concentrations (e.g., Dickerson & Kemeny, 2004; Nicolson, 2007), children were instructed not to eat, drink, or be physically active 30 min prior to the school visit. In these visits, buccal cheek swabs were collected, and children participated in the Children's Reactions to Evaluation Stress Test (CREST; de Weerth et al., 2013) to examine cortisol reactivity. The CREST is a social evaluative stress test that is performed in front of a judge. In this test, children carried out three forced-failure tasks containing elements of unpredictability and uncontrollability. After the three tasks, the judge left the room for 5 min to evaluate the child's performance. The total stress test procedure lasted 20 min; 15 min for the tasks and a 5-min anticipation of the judge's evaluation.

TABLE 1 Descriptives of all the study variables

	N	M/%	SD	Range
Confounders				
Child sex (% girls)	193	47.67		
Maternal education (%)	152			
Secondary education		18.4		
Higher education		81.6		
Child BMI at age 10	157	17.25	2.63	10.21–26.58
Predictors				
Cumulative risk score (% present)	153			
0		39.2		
1		35.3		
2		17.6		
3		3.9		
4		3.9		
Cumulative risk score (standardized continuous)	153	−.01	2.82	−3.90 to 13.09
Individual risk variables (% risk present)				
Parental relationship problems	155	18.1		
Maternal mental health	170	15.3		
Death of someone close	155	42.6		
Bullying victimization	172	14.5		
Poor quality of friendships	169	6.5		
Outcome variables (child)				
Telomere length at age 6	146	1.03	0.54	0.27–3.22
Telomere length at age 10	159	1.04	0.48	0.05–2.59
Telomere length at age 6[a]	145	0.00	1.00	−1.46 to 4.07
Telomere length at age 10[a]	156	0.00	1.00	−2.45 to 3.07
Epigenetic age at age 6	138	7.76	0.66	6.04–9.78
Epigenetic age at age 10	158	12.43	1.10	8.59–15.61
Moderator variables (child)				
Cortisol reactivity (AUCg)	136	365.53	137.03	73.80–795.63
Cortisol reactivity (highest peak–lowest baseline[b])	146	0	1	−1.76 to 5.08

Abbreviations: BMI, body mass index; AUCg, area under the curve with respect to the ground.

[a]These are the standardized residuals saved from regressing telomere length on chronological age at the moment of buccal cell collection.

[b]These are the standardized residuals saved from a regression predicting the highest peak cortisol concentration from the lowest baseline cortisol concentration.

When the judge returned, the child was told that (s)he had performed perfectly well, the child received a present, and a thorough debriefing took place. This procedure was followed by a 25-min recovery phase and 25 min of tasks beyond the scope of this paper. Six saliva samples were collected: one sample right before the start of the stress test (C1), and 5 samples after the start of the stress test (i.e., 15 (C2), 25 (C3), 35 (C4), 50 (C5), and 58 (C6) minutes after the start of the test). For more details on the CREST procedure, see de Weerth et al. (2013);

Simons et al., 2017a; and Simons et al. (2019). At child age 7 and 8, mothers were asked to complete questionnaires online. When children were 10 years old, they were visited at home, length and weight were measured, and mothers filled out questionnaires. Buccal cheek swabs were taken 1 h after the beginning of the home visit. During this time period, no food or drinks were consumed, no physical activity was performed, and children were asked to rinse their mouth with water prior to the collection of the buccal samples.

2.3 | Measures

2.3.1 | Telomere length

At age 6 ($M = 6$ years and 20 days, SD = 67 days) and age 10 ($M = 10$ years and 19 days, SD = 122 days), researchers collected buccal swab samples. DNA was extracted from buccal epithelial cells, using QIAamp DNA Mini Kit (Qiagen) and quantified using Quant-iT PicoGreen reagent (Thermo Fisher Scientific). For DNA quantification, samples were diluted in TE buffer and an equal volume of PicoGreen reagent was added prior to fluorescence measurement. DNA was stored at −80°C until telomere length assays.

Telomere length assays were using a real-time PCR protocol adapted from the method originally published by Cawthon ([2002](#)). Briefly, telomere length is expressed as a ratio of telomeric content (T) to a single-copy gene (S). The single-copy gene used in the assay is *36B4*, which encodes a ribosomal phosphoprotein P0 (*RPLP0*). Separate PCR reactions using DNA from the same sample are conducted to quantify telomeric DNA content and *36B4* content. The cycling profile consists of denaturing at 95°C for 15 s and annealing/extending at 60°C for 1 min followed by fluorescence reading, 45 cycles. The final reaction mix for the telomeric DNA quantification contains 1× SYBR Green Master Mix (Qiagen), 0.2 U Uracil Glycosylase (Thermo Fisher Scientific), 0.1 μM forward primer, 0.1 μM reverse primer, and 3 ng DNA in a 20 μl reaction. The reaction mix for *36B4* quantification contains 1× SYBR Green Master Mix, 0.2 U Uracil Glycosylase, 0.3 μM forward primer, 0.5 μM reverse primer, and 3 ng DNA in a 20 μl reaction. The telomere primer sequences are as follows: forward primer 5′CGGTTTGTTTGGGTTTGGGT-TTGGG TTTGGGTTTGGGTT3′; reverse primer 5′GGCTTGCCTT ACCCTTACCCTTAC-CCTTACCCTTACCCT3′. The *36B4* primer sequences are as follows: forward primer 5′CAG CAAGTGG-GAAGGTGTAATCC3′; reverse primer 5′CCC ATTCTATCATCAACGGGTACAA3′.

Telomere length was quantified as the T/S ratio, calculated as $\mathrm{T/S} = \left(\frac{\mathrm{E_T}^{\mathrm{Cq_T}}}{\mathrm{E_S}^{\mathrm{Cq_S}}} \right)^{-1}$, where $\mathrm{E_{T/S}}$ is the efficiency of exponential amplification for reactions targeting the telomere or single-copy gene respectively, and $\mathrm{Cq_{T/S}}$ is the cycle at which a given replicate targeting telomeric content or the single-copy gene reaches the critical threshold of fluorescence quantification. The threshold of detection is chosen such that all samples cross during their exponential phase of amplification. The same threshold was used for all assays (*36B4* and telomere). The efficiency of exponential amplification using a standard-curve generated using the Rotor-Gene Q instrument software (Version 2.1.0). Standard-curves consisted of a series of five 10-fold dilutions of double-stranded oligomers mimicking telomeric or single-copy gene sequences. Oligomers for the telomere standard-curve were 84 bp long and comprised 16 repeats of the canonical telomere sequence in humans (TTAGGG). Oligomers for the single-copy gene standard-curve consisted of a double-stranded oligomers comprising a 75 bp tract of the *36B4* gene. Sequences for oligomer standards are provided in Table [S1](#).

Samples were run in triplicate and the mean telomeric content ($\mathrm{T} = \mathrm{E_T}^{\mathrm{Cq_T}}$) and mean genome copy number ($\mathrm{S} = \mathrm{E_S}^{\mathrm{Cq_S}}$) across replicates was used for calculating the T/S ratio. When the estimated telomeric content or genome copy number of one replicate deviated from the mean telomeric content or mean genome copy number of the remaining two replicates by more than 15%, it was considered an outlier and the mean estimated telomeric content or genome copy number was recalculated using two replicates. In this manner, the average intra-assay variability for reactions targeting telomeric content and genome copy number was 5.82% and 6.68%, respectively.

To control for inter-assay variability, controls samples were run on each plate. Five control samples were run on plates for 6-year samples. To control for time-dependent batch effects, these same five controls, plus 3 additional controls, were run on plates for 10-year samples. For each plate, the estimated telomeric content and estimated genome copy number of each control DNA was divided by the average telomeric content and genome copy number value for the same DNA across all runs to get a normalizing factor for that sample on a given plate. This was done for all controls to get an average normalizing factor for that plate. In this manner, the average inter-assay for $\mathrm{Cq_T}$ values was 1.23% and for $\mathrm{Cq_S}$ values was 1.10%. A subset of 53 6-year samples was rerun on assays of 10-year assays, which were performed approximately 6 months following original assays of 6-year samples. The ICC across this subset of 53 samples was 0.48.

2.3.2 | Epigenetic age

Buccal cell genomic DNA was used to obtain measures of epigenetic age. The EZ DNA Methylation Kit (Zymo Research) was used for bisulfite conversion of genomic DNA. Next, approximately 160 ng of this DNA was processed using the Illumina EPIC array. Data were preprocessed using the minfi package in R (version 3.2.3; R Core Team, [2015](#)) (see for details McEwen et al., [2019](#); McGill et al., [2022](#)). Observations that did not pass minfi quality control were removed ($n = 1$ at age 6). We derived estimates of buccal epithelial cell content for each sample using a reference-based approach (Smith et al., [2011](#)). Observations with low (<55%) buccal cell content were

removed ($n = 2$ at age 6, $n = 2$ at age 10). Epigenetic age estimates were derived from the PedBE clock which comprises 94 CpGs (see McEwen et al., 2019).

2.3.3 | Cortisol reactivity

The CREST stress test, as described in the procedure section, was performed to measure cortisol reactivity. For collection of saliva samples, eye sponges (BD Visispeare) were used. The samples were centrifuged and stored at −25°C until further analysis in the Laboratory of endocrinology of the University Medical Center Utrecht (for details, see Simons et al., 2017b). Samples were excluded if children used medication that could affect their cortisol concentrations ($n = 3$) and when they deviated from the protocol sampling timing ($n = 1$). A paired samples t-test indicated a significant increase from the lowest baseline ($M = 6.06$ nmol/L, SD = 2.70) to the highest peak concentrations ($M = 7.12$ nmol/L, SD = 3.79), $t(141) = −4.41$, $p = .01$, Cohen's $d = .37$ (see Simons et al., 2017a, 2017b). Cortisol reactivity was calculated as the area under the curve with respect to the ground (AUCg) across all six samples: $\text{AUCg} = (C2 + C1) \times 15/2 + (C3 + C2) \times 10/2 + (C4 + C3) \times 10/2 + (C5 + C4) \times 15/2 + (C6 + C5) \times 8/2$. Moreover, another measure of cortisol reactivity was created by saving the standardized residuals of the highest peak cortisol concentration (C3 or C4) regressed on the lowest baseline cortisol concentration (C1 or C2) (Simons et al., 2017a, 2017b, 2019) and subsequently used in sensitivity analyses.

2.3.4 | Stressor exposure

A cumulative risk score (ranging from 0 to 5) was created by summing the following risk variables. If the risk was present, the participant scored 1 on that risk variable and if the risk was absent the participant scored 0.

Parental relationship problems
Parental relationship problems were measured using 5 items from the Vragenlijst Recent Meegemaakte Gebeurtenissen (VRMG; Van de Willige et al., 1985; in English: Recent Life Change Questionnaire). This is a maternal report questionnaire that was filled out at child age 8 and 10. Mothers had to indicate whether certain situations were applicable to them in the past 2 years. The items used for this risk variable were as follows: an important change in the relationship with your partner, a separation from your partner for at least 1 month due to relationship problems, cheating by yourself, cheating by your partner, and divorce. When one or more of the 5

items were answered with "experienced" (at age 8 and/or 10), the risk was considered present.

Maternal mental health problems
Maternal mental health was measured by maternal anxiety and maternal depression at child age 8. The Dutch translation (Van der Ploeg et al., 1981) of the 20-item state anxiety subscale of the State–Trait Anxiety Inventory (STAI; Spielberger, 1983) was used to measure maternal anxiety symptoms. Answers were provided on a 4-point scale. A cutoff score of 40 was chosen to detect clinically significant symptoms for the state scale, based on the original cutoff proposed by the test developer (Spielberger, 1983). The internal consistency of the STAI in this sample was excellent (Cronbach's α = .93).

Maternal depression symptoms were measured with the Dutch translation (Pop et al., 1992) of the Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987). This is a 10-item scale, with answering options ranging from 0 to 3. A cutoff score of 10 or more was used to detect mothers at risk for a (minor) depression, based on recommendations by Cox et al. (1987). The internal consistency of the EPDS in this sample was good (Cronbach's α = .79). When a mother scored 40 or above on the STAI state scale and/or scored 10 or above on the EPDS, the maternal mental health risk was considered present.

Family/friend death
This risk variable was measured through maternal report on 4 items of the VRMG (Van de Willige et al., 1985): death of your partner, death of a family member that was living at your house, death of another family member, and death of a close friend or neighbor. The answering options were "experienced" or "not experienced." The risk was considered present when at least one item was answered with "experienced" (at age 8 or 10).

Bullying victimization
Bullying victimization was measured through a single item on the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997), which was filled out by the mother at child age 8. Mothers had to indicate whether their child had been bullied or tormented by other kids in the past 6 months. The answering options were "not true," "a little true." or "definitely true." When mother answered the item with "a little true" or "definitely true," the risk was considered present.

Poor quality of friendships
This risk variable was derived from two items reported by mother. The first item is part of the self-esteem subscale of the Child Health Questionnaire (CHQ; Waters et al., 2000) and was measured at child age 7. Mothers were asked how satisfied they thought their child had been about his/her

friendships in the past 4 weeks. Answering options were as follows: "very satisfied," "satisfied," "not satisfied and not unsatisfied," "unsatisfied," "very unsatisfied." The second item is part of the Child Behavior Checklist (CBCL 4–18; Achenbach, 1991) and was measured at child age 7 and again at child age 8. Mothers were asked how many good friends their child had at that time (excluding siblings). Answering options were as follows: "none," "1," "2 or 3" or "4 or more." The risk was considered present when mother answered "unsatisfied" or "very unsatisfied" on the CHQ item, or answered "none" on the CBCL item at age 7 and/or 8.

2.3.5 | Covariates

The covariates in this study were cellular aging (i.e., telomere length or epigenetic age) measured at child age 6, child sex, child BMI at age 10, calculated with the formula $BMI = \frac{weight(kg)}{height(m)^2}$, and maternal education level representing the highest form of education mothers had attained at child age 10. These covariates were chosen based on previous research (Beijers, Hartman, et al., 2020; Shalev, Moffitt, et al., 2013).

2.4 | Data analyses

2.4.1 | Data inspection

The data were inspected for biologically impossible values, and one biological impossible value on cortisol reactivity, measured with AUCg, was replaced with a missing value. The data were subsequently inspected for outliers (defined by a score >3 times the standard deviations above or below the mean), and winsorized (i.e., replaced with the value of the mean plus or minus three standard deviations). The following outliers were identified: telomere length age 6 ($n = 2$), telomere length age 10 ($n = 2$), epigenetic age 10 ($n = 2$), cortisol reactivity (AUCg) ($n = 2$), maternal anxiety ($n = 3$), and maternal depression ($n = 3$). Spearman's correlations were calculated. We tested whether a decrease in telomere length and an increase in epigenetic age from ages 6 to 10 could be observed, using a standard paired samples' t test for epigenetic age, and a paired samples' Wilcoxon test due to non-normality for telomere length.

2.4.2 | Missing data

See Table 1 for the missing values per variable. Missing DNA samples were due to non-participation in the school

and/or home visits during which buccal swab collection took place as a consequence of factors such as lack of time, reluctance towards school involvement, and scheduling difficulties. Missing value analysis showed that data could be assumed to be missing completely at random. Missing values for all but the outcome variables (i.e., telomere length at age 10 and epigenetic aging at age 10) were imputed by means of Markov Chain Monte Carlo using the mice package (version 3.13.0; van Buuren & Groothuis-Oudshoorn, 2011) in R (version 4.0.2; R Core Team, 2020). During the process of multiple imputation, problems arose because some items of the VRMG did not contain any variation; all participants scored 0 on these items. Therefore, we first manually imputed the missing values in these items with score 0. Missing values on other variables were imputed using all available data from all other variables.

2.4.3 | Primary analyses

To test the first research question on the association of cumulative risk and cellular aging, two ordinary least squares regression analyses were performed with cumulative risk score as predictor, and telomere length and epigenetic age at child age 10 as outcome variables. To control for variation in exact child ages in months at which buccal samples were collected, the outcome variables were adjusted for age, by regressing telomere length/epigenetic age at age 10 on chronological age in months at the moment of buccal cell collection (Beijers, Daehn, et al., 2020; Beijers, Hartman, et al., 2020; McEwen et al., 2019). The first regression analysis contained an age-adjusted measure of telomere length at child age 10 as the outcome variable (Beijers, Daehn, et al., 2020; Beijers, Hartman, et al., 2020). Within this regression analysis, we controlled for age-adjusted baseline telomere length measured at age 6, child sex, child BMI at age 10, and maternal education level at age 10. Due to problems with the normality assumptions of regression analyses, the measures of telomere length at age 6 and 10 were log transformed. The outcome variable in the second regression analysis was an age-adjusted measure of PedBE scores at age 10 (McEwen et al., 2019). In this analysis, we controlled for age-adjusted baseline PedBE at age 6, child sex, child BMI at age 10, and maternal education level at age 10.

For the second research question, we tested the moderating effect of cortisol reactivity on the association between cumulative risk and cellular aging, by adding the interaction term between cumulative risk score and child cortisol reactivity to the regression analyses just described. All analyses were performed in R (version 4.0.2; R Core Team, 2020).

2.4.4 | Sensitivity analyses

Both pre-registered and non-preregistered sensitivity analyses were conducted. With regard to the former, two issues were addressed: a recalculation of the cumulative risk score as well as a recalculation of the cortisol reactivity index. The original cumulative-risk index score was replaced with a standardized cumulative risk score of the continuous risk measures. This standardized continuous score was created by summing the standardized continuous scores for each risk variable. For maternal mental health, the average of the standardized continuous scores for maternal anxiety and maternal depression was used. The original cortisol reactivity index (cortisol AUCg) was replaced with another commonly used cortisol reactivity measure: the standardized residuals of the highest peak cortisol concentrations regressed on the lowest baseline cortisol concentrations.

Six non-pre-registered sensitivity analyses were performed. In the first, three risk groups with, respectively 0, 1, and 2+ risk exposures were created, following the approach from Shalev, Moffitt, et al. (2013). In the second, the dichotomous individual stressor variables were used as predictors. For the third, missing values in the entire dataset ($n = 193$), now including those in the outcome variables, were imputed and subsequently the primary analyses were repeated. For the fourth, missing data were not imputed. For the fifth, raw instead of age-adjusted values were used for our measures of cellular aging (telomere length and epigenetic age at age 6 and 10). For the sixth, we repeated the primary analyses, without controlling for baseline measures of telomere length and epigenetic age at age 6. Some literature suggests that including baseline measures might bias the association between variables, if the independent variable is already present prior to, and associated with, baseline measurement (Bateson et al., 2019; Glymour et al., 2005). In this study, not all, but some of the risks, could have been present prior to age 6. Therefore, this last analysis was included to prevent possible over-adjustment.

3 | RESULTS

3.1 | Descriptive analyses

Table 2 displays Spearman's correlations between the study variables. While the measures of telomere length at age 6 and age 10 were not significantly correlated ($p = .107$), older epigenetic age at the first measurement occasion was associated with older epigenetic age several years later ($p < .001$). No significant correlations emerged between the cumulative risk scores and cellular aging at

age 10: telomere length at age 10 ($p = .574$), and epigenetic aging at age 10 ($p = .812$). Greater cumulative risk was correlated to lower cortisol reactivity, as measured with the standardized residuals of the regression of the highest peak on the lowest baseline ($p = .018$). Finally, longer telomere length at age 6 was associated with greater cortisol reactivity at age 6 (AUCg: $p = .003$; highest peak ~ lowest baseline: $p = .042$).

As expected, the paired samples' test indicated that average epigenetic age increased from age 6 to 10 ($p < .001$). Unexpectedly, a decrease in average telomere length from age 6 to 10 was not observed ($p = .360$; for age-adjusted telomere length $p = .610$).

3.2 | Primary analyses

As can be seen in Table 3, the regression analysis indicated that cumulative risk score was not associated with change in telomere length ($p = .491$) nor in epigenetic age ($p = .281$) from age 6 to 10. In addition, the tested interaction between cumulative risk and cortisol reactivity was not significant in predicting change in telomere length ($p = .445$) or epigenetic age ($p = .885$).

3.3 | Sensitivity analyses

Similar to results of the primary analyses, all sensitivity analyses addressing the two core questions of this inquiry proved insignificant. That is, results were the same as already summarized, though regression coefficients and p values were slightly different.

4 | DISCUSSION

4.1 | Main findings

The aim of this study was to investigate the association between childhood stress exposure and cellular aging in a low-risk, Dutch community sample. In addition, the possible moderating effect of cortisol reactivity was examined based on ideas about differential susceptibility to environmental influences. Contrary to expectations, results did not reveal significant associations between cumulative risk exposure and change in either of the biomarkers of cellular aging, that is, telomere length and epigenetic aging. Neither did we find evidence that highly physiologically reactive children proved more susceptible to effects of childhood risk on cellular aging. This was the case independent of the specific parameterizations of the core constructs.

TABLE 2 Spearman correlations between study variables

	1	2	3	4	5	6	7	8	9	10	11	12	13
Outcome variables													
1. Telomere length age 10													
2. Epigenetic age 10	−.07												
Predictors													
3. Parent relationship problems	−.02	.08											
4. Maternal mental health	−.05	−.11	.30**										
5. Death someone close	.09	−.06	.04	.08									
6. Bullying victimization	−.07	−.06	.13	.29**	.12								
7. Poor quality friendships	.04	.07	.09	.09	.04	.09							
8. Cumulative risk score (regular)	.05	.02	.54**	.55**	.64**	.52**	.30**						
9. Cumulative risk score (standardized continuous)	−.01	.09	.50**	.44***	.47**	.50***	.29**	.82**					
Moderators													
10. Cortisol reactivity (AUCg)	.01	.05	−.14	−.05	.08	.00	.01	−.05	.01				
11. Cortisol reactivity (standardized residuals)	.10	−.04	−.13	−.08	−.13	.01	−.12	−.20*	−.15	.45**			
Controls													
12. Telomere length age 6	.14	.03	−.03	−.09	.02	−.04	−.01	−.05	−.03	.25***	.17*		
13. Epigenetic age 6	−.14	.59**	.00	−.04	.03	−.02	.02	.04	.03	−.04	−.13	.13	

*$p<.05$; **$p<.001$.

	TL at age 10 (log)		PedBE at age 10	
	b	**SE**	**b**	**SE**
Main analyses				
Cumulative risk	0.02	0.03	−0.07	0.06
TL (log)/PedBE at age 6	0.13	0.10	0.99**	0.10
Child sex (boy)	−0.01	0.05	−0.32*	0.14
Child BMI	0.01	0.01	−0.02	0.04
Maternal education level	0.02	0.02	0.03	0.05
Model fit (R^2)	.02		.44	
Moderation analyses				
Cumulative risk	0.02	0.03	−0.07	0.06
Cortisol reactivity (AUCg)	0.00	0.00	0.00	0.00
TL (log)/PedBE at age 6	0.14	0.11	1.00**	0.10
Child sex	−0.01	0.06	−0.29*	0.14
Child BMI	0.00	0.01	−0.02	0.04
Maternal education level	0.02	0.02	0.04	0.05
Cumulative risk × cortisol reactivity (AUCg)	0.00	0.00	0.00	0.00
Model fit (R^2)	.03		.45	
Sensitivity analyses				
Standardized cumulative risk				
Main effect	0.00	0.01	−0.00	0.02
Standardized cumulative risk × cortisol reactivity (AUCg)	0.00	0.00	0.00	0.00
Individual stressors				
Parental relationship problems	−0.02	0.07	0.15	0.17
Maternal mental health	0.02	0.08	−0.25	0.18
Death of someone close	0.08	0.05	−0.18	0.13
Bullying victimization	−0.03	0.08	−0.16	0.18
Poor quality of friendships	0.06	0.11	0.00	0.27
Cortisol reactivity (highest peak–lowest baseline)				
Cumulative risk × cortisol reactivity (highest peak–lowest baseline)	0.04	0.03	0.02	0.06
Standardized cumulative risk × cortisol reactivity (highest peak–lowest baseline)	0.01	0.01	0.01	0.03

TABLE 3 Ordinary least squares regression models for the prediction of the two biomarkers of cellular aging and relevant coefficients for sensitivity analyses

Abbreviations: AUCg, area under the curve with respect to the ground; TL, telomere length; PedBE, pediatric-buccal-epigenetic clock.

**p < .001; *p < .05.

It is important to appreciate that absence of evidence is not evidence of absence. Our results are not in line with much research indicating, or at least suggesting, that the telomeres of children exposed to (cumulative) childhood risks erode faster (Pepper et al., 2018; Ridout et al., 2018). They also diverge from previous studies documenting links between greater childhood stress exposure and accelerated epigenetic aging (Jovanovic et al., 2017; Marini et al., 2020; Sumner et al., 2019), although it should be noted that this is the first study to use the PedBE clock. Furthermore, previous research on the effects of stress on child cellular aging has produced

(some) evidence consistent with differential susceptibility theory (Beijers, Hartman, et al., 2020; Mitchell et al., 2014). Thus, there is support for the theory in other studies.

A possible reason for the reported null findings in the face of prior evidence was our focus on a low-risk community sample, in contrast to most previous studies (e.g., Drury et al., 2014; Jovanovic et al., 2017; Ridout et al., 2019). It could well be the case that levels of adversity must be higher than what our sample experienced in order to discern accelerated-aging effects. And this may be especially so with respect to the kinds of risk assessed, as other studies documenting stress-related effects on accelerated aging have focused on more severe stressors, such as family violence (e.g., Shalev, Moffitt, et al., 2013) and severe social deprivation (e.g., Drury et al., 2014). Also worth considering is that in our sample effects of stress exposure may have been buffered by other factors, such as a sensitive parent, a harmonious marriage, supportive sibling relations and/or supportive teachers. It is, for example, known that the effects of stress on cellular aging are socially buffered by secure attachments (Dagan et al., 2018; Ehrlich et al., 2021). Recall that our low-risk community sample was comprised mostly of highly educated parents, with most children having at least one good friend. All these factors may buffer the effects of childhood risks.

Another possible explanation of why this study did not reveal evidence for the hypotheses has to do with the nature of the risks. A meta-analysis performed by Colich et al. (2020) after we registered our study plan found that threat-related adversity (e.g., violence exposure) was associated with accelerated cellular aging, whereas deprivation-related adversity (e.g., neglect), including low SES, was not. The lack of threat-related risk indicators in our research could be a reason why we did not discern any association between childhood stress exposure and cellular aging. Indeed, the only risk that could be considered threat-related would be bullying victimization. The non-preregistered sensitivity analysis with a focus on bullying as a threat-related indicator showed, however, no evidence that this indicator predicted cellular aging.

4.2 | Secondary findings

In addition to the findings that were directly related to our research questions, other interesting findings appear worth discussing. First, we found that the two cellular aging measures at age 10 were not correlated to each other. These results are in line with previous research in adults which has shown that correlations between telomere length/erosion, epigenetic clocks, and biomarker composite scores are generally low (Belsky et al., 2018),

possibly because each of these is measuring a distinct part of biological aging.

Second, we did not observe a decrease in average telomere length from age 6 to 10. Although this finding is unexpected, a recent review suggests that telomere length might be stable for some years after the first 3 years of life, before gradually decreasing again (Gorenjak et al., 2020). Indeed, a recent longitudinal study, not included in the aforementioned review, also reports of period of stability between the first 3 years of life and early adolescence (Cowell et al., 2021).

Third, shorter telomeres at age 6 were associated with blunted levels of cortisol reactivity at age 6. This finding stands in contrast to that of Gotlib et al. (2015) who showed that girls (aged 10–14) with shorter telomeres had *greater* cortisol reactivity to stress. Possibly, the contradictory findings could be explained by research findings in adults, which indicate that early life stress can be followed by multiple atypical patterns of HPA-axis reactivity, including both elevated and blunted cortisol responses (Boyce & Ellis, 2005; van Bodegom et al., 2017). However, more longitudinal studies with multiple time points across childhood are necessary to elucidate the associations between physiological measures of stress and cellular aging.

4.3 | Strengths and limitations

The current study has both strengths and limitations. Reflecting the former are a prospective research design with regard to stress effects on cellular aging; a focus on a low-risk sample; reliance on a pediatric-specific epigenetic clock; and consideration of multiple parameterizations of core constructs. Turning to limitations, measurements of telomere length and epigenetic aging were derived from buccal epithelial cells instead of blood cells, which are more commonly used (Demanelis et al., 2020). Nonetheless, several studies suggest that telomere length is correlated across multiple tissues (Daniali et al., 2013; Demanelis et al., 2020; Gadalla et al., 2010). Secondly, assays to estimate telomere length for 6 years samples were performed in an independent batch than assays to estimate telomere length for 10 years samples. Although both assays followed the same established protocol, the ICC of replication of 6 years samples stored and run on both batches was 0.48, indicating moderate reproducibility. While this deviance could be attributed to DNA degradation in the interim between assays, it could also be indicative of batch effects that introduce error and contribute to null findings. A third limitation concerns the predictor measurement. Following prior investigations (e.g., Shalev, Moffitt, et al., 2013), five individual risk indicators were composited to create an index of cumulative risk. The

downside to this approach is that it assumes equal weighting of adversities and specific combinations of adversities are ignored (Cohodes et al., 2021). Perhaps even more limiting is that there are many other adverse conditions that might have been added to our five "suspects" or used instead of some of them. Lastly, one of the main strengths of this study—the reliance on a low-risk sample—can also be regarded as a weakness.

4.4 | Conclusion

In conclusion, the present study shows that the association between childhood stressor exposure and cellular aging might only be present in high-adversity risk (community) samples, not in low-adversity risk community samples. Since this is the first study examining these associations, more research is necessary to find out whether there truly is no association between a cumulative pattern of mild childhood risks and change in cellular aging in community samples, or whether it is just not found in our specific low-risk community sample. In addition to the importance of specific types and severity of stressors, as well as when and how children are affected by stressor exposure, it is important to know which children are most susceptible to the possible negative consequences following stressor exposure to inform future development of intervention and prevention strategies.

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REFERENCES
Achenbach, T. M. (1991). *Manual for the Child Behavior Checklist/4–18 and 1991 profile*. University of Vermont, Department of Psychiatry.

Alisch, R. S., Barwick, B. G., Chopra, P., Myrick, L. K., Satten, G. A., Conneely, K. N., & Warren, S. T. (2012). Age-associated DNA methylation in pediatric populations. *Genome Research*, *22*(4), 623–632. https://doi.org/10.1101/gr.125187.11

Asok, A., Bernard, K., Roth, T. L., Rosen, J. B., & Dozier, M. (2013). Parental responsiveness moderates the association between early-life stress and reduced telomere length. *Development and Psychopathology*, *25*, 577–585. https://doi.org/10.1017/S0954579413000011

Bateson, M., Eisenberg, D. T., & Nettle, D. (2019). Controlling for baseline telomere length biases estimates of the rate of telomere attrition. *Royal Society Open Science*, *6*(10), 190937. https://doi.org/10.1098/rsos.190937

Beijers, R., Daehn, D., Shalev, I., Belsky, J., & de Weerth, C. (2020). Biological embedding of maternal postpartum depressive symptoms: The potential role of cortisol and telomere length. *Biological Psychology*, *150*, 107809. https://doi.org/10.1016/j.biopsycho.2019.107809

Beijers, R., Hartman, S., Shalev, I., Hastings, W., Mattern, B. C., de Weerth, C., & Belsky, J. (2020). Testing three hypotheses about effects of sensitive–insensitive parenting on telomeres. *Developmental Psychology*, *56*(2), 237–250. https://doi.org/10.1037/dev0000879

Beijers, R., Jansen, J., Riksen-Walraven, M., & de Weerth, C. (2011). Attachment and infant night waking: A longitudinal study from birth through the first year of life. *Journal of Developmental & Behavioral Pediatrics*, *32*(9), 635–643. https://doi.org/10.1542/peds.2009-3226

Belsky, D. W., Moffitt, T. E., Cohen, A. A., Corcoran, D. L., Levine, M. E., Prinz, J. A., Schaefer, J., Sugden, K., Williams, B., Poulton,

R., & Caspi, A. (2018). Eleven telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: Do they measure the same thing? *American Journal of Epidemiology*, *187*(6), 1220–1230. https://doi.org/10.1093/aje/kwx346

Belsky, J. (1997). Variation in susceptibility to rearing influence: An evolutionary argument. *Psychological Inquiry*, *8*, 182–186. https://doi.org/10.1207/s15327965pli0803_3

Belsky, J. (2005). Differential susceptibility to rearing influences: An evolutionary hypothesis and some evidence. In B. Ellis & D. Bjorklund (Eds.), *Origins of the social mind: Evolutionary psychology and child development* (pp. 139–163). Guildford.

Belsky, J., Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (2007). For better and for worse: Differential susceptibility to environmental influences. *Current Directions in Psychological Science*, *16*(6), 300–304. 10.1111%2Fj.1467-8721.2007.00525.x

Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: Differential susceptibility to environmental influences. *Psychological Bulletin*, *135*(6), 885–908. https://doi.org/10.1037/a0017376

Belsky, J., & Pluess, M. (2013). Beyond risk, resilience, and dysregulation: Phenotypic plasticity and human development. *Development and Psychopathology*, *25*(4pt2), 1243–1261. https://doi.org/10.1017/S095457941300059X

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary–developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology*, *17*(2), 271–301. https://doi.org/10.1017/S0954579405050145

Campisi, J., & d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. *Nature Reviews Molecular Cell Biology*, *8*(9), 729–740. https://doi.org/10.1038/nrm2233.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research*, *30*(10), e47. https://doi.org/10.1093/nar/30.10.e47

Chen, B. H., Marioni, R. E., Colicino, E., Peters, M. J., Ward-Caviness, C. K., Tsai, P. C., Roetker, N. S., Just, A. C., Demerath, E. W., Guan, W., Bressler, J., Fornage, M., Studenski, S., Vandiver, A. R., Moore, A. Z., Tanaka, T., Kiel, D. P., Liang, L., Vokonas, P., ... Horvath, S. (2016). DNA methylation-based measures of biological age: Meta-analysis predicting time to death. *Aging*, *8*(9), 1844–1865. https://doi.org/10.18632/aging.101020

Cohodes, E. M., Kitt, E. R., Baskin-Sommers, A., & Gee, D. G. (2021). Influences of early-life stress on frontolimbic circuitry: Harnessing a dimensional approach to elucidate the effects of heterogeneity in stress exposure. *Developmental Psychobiology*, *63*(2), 153–172. https://doi.org/10.1002/dev.21969

Colich, N. L., Rosen, M. L., Williams, E. S., & McLaughlin, K. A. (2020). Biological aging in childhood and adolescence following experiences of threat and deprivation: A systematic review and meta-analysis. *Psychological Bulletin*, *146*(9), 721–764. https://doi.org/10.1037/bul0000270

Cowell, W., Tang, D., Yu, J., Guo, J., Wang, S., Baccarelli, A. A., Perera, F., & Herbstman, J. B. (2021). Telomere dynamics across the early life course: Findings from a longitudinal study in children. *Psychoneuroendocrinology*, *129*, 105270.

Cox, J. L., Holden, J. M., & Sagovsky, R. (1987). Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. *The British Journal of Psychiatry*, *150*(6), 782–786. https://doi.org/10.1192/BJP.150.6.782

Dagan, O., Asok, A., Steele, H., Steele, M., & Bernard, K. (2018). Attachment security moderates the link between adverse childhood experiences and cellular aging. *Development and Psychopathology*, *30*(4), 1211–1223. https://doi.org/10.1017/S0954579417001705

Daniali, L., Benetos, A., Susser, E., Kark, J. D., Labat, C., Kimura, M., Desai, K. K., Granick, M., & Aviv, A. (2013). Telomeres shorten at equivalent rates in somatic tissues of adults. *Nature Communications*, *4*(1), 1–7. https://doi.org/10.1038/ncomms2602

de Weerth, C., Zijlmans, M. A. C., Mack, S., & Beijers, R. (2013). Cortisol reactions to a social evaluative paradigm in 5-and 6-year-old children. *Stress*, *16*, 65–72. https://doi.org/10.3109/10253890.2012.684112

Demanelis, K., Jasmine, F., Chen, L. S., Chernoff, M., Tong, L., Delgado, D., Zhang, C., Shinkle, J., Sabarinathan, M., Lin, H., Ramirez, E., Oliva, M., Kim-Hellmuth, S., Stranger, B. E., Ardlie, K. G., Aguet, F., Ahsan, H., GTEx Consortium, Doherty, J., ... Pierce, B. L. (2020). Determinants of telomere length across human tissues. *Science*, *369*(6509), eaaz687. https://doi.org/10.1126/science.aaz6876

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*, 355–391. https://doi.org/10.1037/0033-2909.130.3.355

Drury, S. S., Mabile, E., Brett, Z. H., Esteves, K., Jones, E., Shirtcliff, E. A., & Theall, K. P. (2014). The association of telomere length with family violence and disruption. *Pediatrics*, *134*, 128–137. https://doi.org/10.1542/peds.2013-3415

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y. Y., Fox, N. A., Zeanah, C. H., & Nelson, C. A. (2012). Telomere length and early severe social deprivation: Linking early adversity and cellular aging. *Molecular Psychiatry*, *17*, 719–727. https://doi.org/10.1038/mp.2011.53

Ehrlich, K. B., Yu, T., Sadiq, A., & Brody, G. H. (2021). Neighborhood poverty, allostatic load, and changes in cellular aging in African American young adults: The moderating role of attachment. *Attachment & Human Development*, *1-14*, 339–352. https://doi.org/10.1080/14616734.2021.1976934

Ellis, B. J., Boyce, W. T., Belsky, J., Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (2011). Differential susceptibility to the environment: An evolutionary–neurodevelopmental theory. *Development and Psychopathology*, *23*(1), 7–28. https://doi.org/10.1017/S0954579410000611

Forero, D. A., González-Giraldo, Y., López-Quintero, C., Castro-Vega, L. J., Barreto, G. E., & Perry, G. (2016). Meta-analysis of telomere length in Alzheimer's disease. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *71*(8), 1069–1073. https://doi.org/10.1093/gerona/glw053

Gadalla, S. M., Cawthon, R., Giri, N., Alter, B. P., & Savage, S. A. (2010). Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes. *Aging*, *2*(11), 867–874. https://doi.org/10.18632/aging.100235

Glymour, M. M., Weuve, J., Berkman, L. F., Kawachi, I., & Robins, J. M. (2005). When is baseline adjustment useful in analyses of change? An example with education and cognitive change. *American Journal of Epidemiology*, *162*(3), 267–278. https://doi.org/10.1093/aje/kwi187

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: A research note. *Journal of Child Psychology and Psychiatry*, *38*(5), 581–586. https://doi.org/10.1111/j.1469-7610.1997.tb01545.x

Gorenjak, V., Petrelis, A., Stathopoulou, M., & Visvikis-Siest, S. (2020). Telomere length determinants in childhood. *Clinical Chemistry and Laboratory Medicine*, *58*(2), 162–177. https://doi.org/10.1515/cclm-2019-0235

Gotlib, I. H., LeMoult, J., Colich, N. L., Foland-Ross, L. C., Hallmayer, J., Joormann, J., Lin, J., & Wolkowitz, O. M. (2015). Telomere length and cortisol reactivity in children of depressed mothers. *Molecular Psychiatry*, *20*(5), 615–620. https://doi.org/10.1038/mp.2014.119

Hardt, J., & Rutter, M. (2004). Validity of adult retrospective reports of adverse childhood experiences: Review of the evidence. *Journal of Child Psychology and Psychiatry*, *45*(2), 260–273. https://doi.org/10.1111/j.1469-7610.2004.00218.x

Haycock, P. C., Heydon, E. E., Kaptoge, S., Butterworth, A. S., Thompson, A., & Willeit, P. (2014). Leucocyte telomere length and risk of cardiovascular disease: Systematic review and meta-analysis. *BMJ*, *349*, g4227. https://doi.org/10.1136/bmj.g4227

Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biology*, *14*(10), 1–20. https://doi.org/10.1186/gb-2013-14-10-r115

Horvath, S., & Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews Genetics*, *19*(6), 371–384. https://doi.org/10.1038/s41576-018-0004-3

Humphreys, K. L., Esteves, K., Zeanah, C. H., Fox, N. A., Nelson, C. A., III, & Drury, S. S. (2016). Accelerated telomere shortening: Tracking the lasting impact of early institutional care at the cellular level. *Psychiatry Research*, *246*, 95–100. https://doi.org/10.1016/j.psychres.2016.09.023

Jovanovic, T., Vance, L. A., Cross, D., Knight, A. K., Kilaru, V., Michopoulos, V., Klengel, T., & Smith, A. K. (2017). Exposure to violence accelerates epigenetic aging in children. *Scientific Reports*, *7*(1), 1–7. https://doi.org/10.1038/s41598-017-09235-9

Kelly-Irving, M., Lepage, B., Dedieu, D., Lacey, R., Cable, N., Bartley, M., Blane, D., Grosclaude, P., Lang, T., & Delpierre, C. (2013). Childhood adversity as a risk for cancer: Findings from the 1958 British birth cohort study. *BMC Public Health*, *13*(1), 1–13. https://doi.org/10.1186/1471-2458-13-767

Kiecolt-Glaser, J. K., Gouin, J. P., Weng, N. P., Malarkey, W. B., Beversdorf, D. Q., & Glaser, R. (2011). Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosomatic Medicine*, *73*(1), 16–22. https://doi.org/10.1097/PSY.0b013e31820573b6

Ma, H., Zhou, Z., Wei, S., Liu, Z., Pooley, K. A., Dunning, A. M., Svenson, U., Roos, G., Hosgood, H. D., III, Shen, M., & Wei, Q. (2011). Shortened telomere length is associated with increased risk of cancer: A meta-analysis. *PLoS One*, *6*(6), e20466. https://doi.org/10.1371/journal.pone.0020466

Marini, S., Davis, K. A., Soare, T. W., Zhu, Y., Suderman, M. J., Simpkin, A. J., Smith, A. D., Wolf, E. J., Relton, C. L., & Dunn, E. C. (2020). Adversity exposure during sensitive periods predicts accelerated epigenetic aging in children. *Psychoneuroendocrinology*, *113*, 104484. https://doi.org/10.1016/j.psyneuen.2019.104484

McEwen, L. M., O'Donnell, K. J., McGill, M. G., Edgar, R. D., Jones, M. J., MacIsaac, J. L., Lin, D. T. S., Ramadori, K., Morin, A., Gladish, N., Garg, E., Unternaehrer, E., Pokhvisneva, I., Karnani, N., Kee, M. Z. L., Klengel, T., Adler, N. E., Barr, R. G., Letourneau, N., ... Kobor, M. S. (2019). The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(38), 23329–23335. https://doi.org/10.1073/pnas.1820843116

McGill, M. G., Pokhvisneva, I., Clappison, A. S., McEwen, L. M., Beijers, R., Tollenaar, M. S., Pham, H., Kee, M. Z., Garg, E., de Mendonça Filho, E. J., Karnani, N., Silveira, P. P., Kobor, M. S., de Weerth, C., Meaney, M. J., & O'Donnell, K. J. (2022). Maternal prenatal anxiety and the fetal origins of epigenetic aging. *Biological Psychiatry*, *91*(3), 303–312. https://doi.org/10.1016/j.biopsych.2021.07.025

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., Garfinkel, I., & Notterman, D. (2014). Social disadvantage, genetic sensitivity, and children's telomere length. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16), 5944–5949. https://doi.org/10.1073/pnas.1404293111

Monroe, S. M., & Simons, A. D. (1991). Diathesis–stress theories in the context of life stress research. *Psychological Bulletin*, *110*(3), 406–425. https://doi.org/10.1037/0033-2909.110.3.406

Needham, B. L., Fernandez, J. R., Lin, J., Epel, E. S., & Blackburn, E. H. (2012). Socioeconomic status and cell aging in children. *Social Science & Medicine*, *74*(12), 1948–1951. https://doi.org/10.1016/j.socscimed.2012.02.019

Nicolson, N. A. (2007). Measurement of cortisol. In L. J. Luecken & L. C. Gallo (Eds.), *Handbook of physiological research methods in health psychology* (pp. 37–74). Sage Publications. https://doi.org/10.4135/9781412976244.n3

Pepper, G. V., Bateson, M., & Nettle, D. (2018). Telomeres as integrative markers of exposure to stress and adversity: A systematic review and meta-analysis. *Royal Society Open Science*, *5*(8), 180744. https://doi.org/10.1098/rsos.180744

Pierce, J. B., Kershaw, K. N., Kiefe, C. I., Jacobs, D. R., Jr., Sidney, S., Merkin, S. S., & Feinglass, J. (2020). Association of childhood psychosocial environment with 30-year cardiovascular disease incidence and mortality in middle age. *Journal of the American Heart Association*, *9*(9), e015326. https://doi.org/10.1161/JAHA.119.015326

Pirkola, S., Isometsä, E., Aro, H., Kestilä, L., Hämäläinen, J., Veijola, J., Kiviruusu, O., & Lönnqvist, J. (2005). Childhood adversities as risk factors for adult mental disorders. *Social Psychiatry and Psychiatric Epidemiology*, *40*(10), 769–777. https://doi.org/10.1007/s00127-005-0950-x

Pop, V. J., Komproe, I. H., & Van Son, M. J. (1992). Characteristics of the Edinburgh post natal depression scale in The Netherlands. *Journal of Affective Disorders*, *26*(2), 105–110. https://doi.org/10.1016/0165-0327(92)90041-4

R Core Team (2015). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Repetti, R. L., Taylor, S. E., & Seeman, T. E. (2002). Risky families: Family social environments and the mental and physical health of offspring. *Psychological Bulletin*, *128*(2), 330–366. https://doi.org/10.1037/0033-2909.128.2.330

Ridout, K. K., Levandowski, M., Ridout, S. J., Gantz, L., Goonan, K., Palermo, D., Price, L. H., & Tyrka, A. R. (2018). Early life adversity and telomere length: A meta-analysis. *Molecular Psychiatry*, *23*(4), 858–871. https://doi.org/10.1038/mp.2017.26

Ridout, K. K., Parade, S. H., Kao, H. T., Magnan, S., Seifer, R., Porton, B., Price, L. H., & Tyrka, A. R. (2019). Childhood maltreatment, behavioral adjustment, and molecular markers

of cellular aging in preschool-aged children: A cohort study. *Psychoneuroendocrinology*, *107*, 261–269. https://doi.org/10.1016/j.psyneuen.2019.05.015

Shalev, I. (2012). Early life stress and telomere length: Investigating the connection and possible mechanisms: A critical survey of the evidence base, research methodology and basic biology. *BioEssays*, *34*(11), 943–952. https://doi.org/10.1002/bies.201200084

Shalev, I., Entringer, S., Wadhwa, P. D., Wolkowitz, O. M., Puterman, E., Lin, J., & Epel, E. S. (2013). Stress and telomere biology: A lifespan perspective. *Psychoneuroendocrinology*, *38*(9), 1835–1842. https://doi.org/10.1016/j.psyneuen.2013.03.010

Shalev, I., Moffitt, T. E., Sugden, K., Williams, B., Houts, R. M., Danese, A., Mill, J., Arseneault, L., & Caspi, A. (2013). Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: A longitudinal study. *Molecular Psychiatry*, *18*, 576–581. https://doi.org/10.1038/mp.2012.32

Simons, S. S., Zijlmans, M. A., Cillessen, A. H., & de Weerth, C. (2019). Maternal prenatal and early postnatal distress and child stress responses at age 6. *Stress*, *22*(6), 654–663. https://doi.org/10.1080/10253890.2019.1608945

Simons, S. S. H., Cillessen, A. H. N., & de Weerth, C. (2017a). Associations between circadian and stress response cortisol in children. *Stress*, *20*, 69–75. https://doi.org/10.1080/10253890.2016.1276165

Simons, S. S. H., Cillessen, A. H. N., & de Weerth, C. (2017b). Cortisol stress responses and children's behavioral functioning at school. *Developmental Psychobiology*, *59*, 217–224. https://doi.org/10.1002/dev.21484

Smith, A. K., Conneely, K. N., Kilaru, V., Mercer, K. B., Weiss, T. E., Bradley, B., Tang, Y., Gillespie, C. F., Cubells, J. F., & Ressler, K. J. (2011). Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *156*(6), 700–708. https://doi.org/10.1002/ajmg.b.31212

Spielberger, C. D. (1983). *Manual for the State-trait anxiety inventory for adults*. Consulting Psychologists Press.

Sumner, J. A., Colich, N. L., Uddin, M., Armstrong, D., & McLaughlin, K. A. (2019). Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. *Biological Psychiatry*, *85*, 268–278. https://doi.org/10.1016/j.biopsych.2018.09.008

van Bodegom, M., Homberg, J. R., & Henckens, M. J. (2017). Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. *Frontiers in Cellular Neuroscience*, *11*, 1–33. https://doi.org/10.3389/fncel.2017.00087

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, *45*(3), 1–67. www.jstatsoft.org/v45/i03/

Van de Willige, G., Schreurs, P., Tellegen, B., & Zwart, F. (1985). Het meten van "life events": de Vragenlijst Recent Meegemaakte Gebeurtenissen (VRMG). *Nederlands Tijdschrift voor de Psychologie en haar Grensgebieden*, *40*(1), 1–19.

Van der Ploeg, H. M., Defares, P. B., & Spielberger, C. D. (1981). *Een Nederlandstalige bewerking van de Spielberger State – Trait Anxiety Inventory [A Dutch adaptation of the Spilberger's State – Trait anxiety inventory]*. Swets and Zeitlinger.

Waters, E., Salmon, L., Wake, M., Hesketh, K., & Wright, M. (2000). The Child Health Questionnaire in Australia: Reliability, validity and population means. *Australian and New Zealand Journal of Public Health*, *24*(2), 207–210. https://doi.org/10.1111/j.1467-842X.2000.tb00145.x

Wentzensen, I. M., Mirabello, L., Pfeiffer, R. M., & Savage, S. A. (2011). The association of telomere length and cancer: A meta-analysis. *Cancer Epidemiology and Prevention Biomarkers*, *20*(6), 1238–1250. https://doi.org/10.1158/1055-9965.EPI-11-0005

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.