No Mediation Effect of Telomere Length or Mitochondrial DNA Copy Number on the Association Between Adverse Childhood Experiences (ACEs) and Central Arterial Stiffness

Nathaniel J. Iannarelli, MSc; Terrance J. Wade, PhD; Kylie S. Dempster, PhD; Jessy Moore, MSc; Adam J. MacNeil, PhD; Deborah D. O’Leary, PhD

BACKGROUND: Adverse childhood experiences (ACEs) have been linked to increased cardiovascular disease (CVD) risk. Previous reports have suggested that accelerated biological aging—indexed by telomere length (TL) and mitochondrial DNA copy number (mtDNAcn)—may contribute to associations between ACEs and cardiovascular health outcomes. Here, we examine the potential mediating effects of TL and mtDNAcn on the association between ACEs and central arterial stiffness—an intermediate cardiovascular health outcome—as a novel pathway linking ACEs to CVD risk among young adults.

METHODS AND RESULTS: One hundred and eighty-five (n=102 women; mean age, 22.5±1.5 years) individuals provided information on ACEs. TL (kb per diploid cell) and mtDNAcn (copies per diploid cell) were quantified using quantitative polymerase chain reaction techniques. Central arterial stiffness was measured as carotid-femoral pulse wave velocity (cfPWV; m/s). Multiple linear regression analyses were used to examine the associations between ACEs, TL, mtDNAcn, and cfPWV. ACEs were positively associated with cfPWV (β=0.147, P=0.035). TL (β=−0.170, P=0.011) and mtDNAcn (β=−0.159, P=0.019) were inversely associated with cfPWV. Neither TL (β=−0.027, P=0.726) nor mtDNAcn (β=0.038, P=0.620) was associated with ACEs. Neither marker mediated the association between ACEs and cfPWV.

CONCLUSIONS: An increasing number of ACEs were associated with a faster cfPWV and thus, a greater degree of central arterial stiffness. ACEs were not associated with either TL or mtDNAcn, suggesting that these markers do not represent a mediating pathway linking ACEs to central arterial stiffness.

Key Words: adverse childhood experiences ▪ biological aging ▪ central arterial stiffness ▪ mitochondrial DNA copy number ▪ telomere length ▪ vascular aging
Canadian adults had experienced ≥1 ACE,15 which is of importance as ACEs tend to co-occur.12,18,19 Moreover, ACEs have been consistently linked to higher rates of mental health disorders (eg, post-traumatic stress disorder), common chronic diseases (eg, CVD),20,21 and higher health care costs.20

The landmark ACE study,12 first reported a positive graded association between ACEs and CVD (specifically, ischemic heart disease) risk.10 Since then, a growing body of literature has bolstered this finding, demonstrating the lasting and profound effect of ACEs on cardiovascular health and function.14,22 Despite these findings, the underlying mechanisms linking ACEs to cardiovascular health outcomes remain unclear. Accumulating evidence suggests that accelerated biological aging may be one pathway linking ACEs and negative health outcomes.23–25 With respect to cardiovascular health, ACEs may increase CVD risk through their impact on the structure and function of the central arteries (ie, the aorta and its major branches).12,26,27 Previous studies have found evidence of increased arterial stiffness27–30 and common carotid artery (CCA) intima media thickness31,32 among individuals reporting high levels of childhood adversity, which suggests a susceptibility of the arterial vasculature to undergo deleterious remodeling in response to ACEs. However, it remains unclear if ACEs are associated with increased central arterial stiffness, as measured by the carotid-femoral pulse wave velocity (cfPWV; “gold standard” measure of arterial stiffness33–35), in young adults free of overt CVD, and by what mechanism(s) ACEs may confer increased arterial stiffness.

Mounting evidence suggests that ACEs may “get under the skin” and influence cardiovascular health, in part, via dysregulation of the stress response systems (ie, the hypothalamic–pituitary–adrenal axis, sympathetic nervous system, and immune system).13,36–38 Indeed, maladaptive metabolic and immune responses have been implicated as potential mediators underpinning the ACEs-cardiovascular health relationship.36,37 Specifically, childhood adversity disrupts allostasis, leading to physiological dysfunction across the stress response systems, resulting in elevated biological stress (ie, oxidative and inflammatory stress) via downstream metabolic and inflammatory pathways.13,36–38 Elevated biological stress is thought to promote accelerated biological and vascular aging, which is associated with increased central arterial stiffness and CVD risk.39–42 Here, we examine 2 indices of biological aging as potential mediators linking ACEs to increased arterial stiffness—telomere length (TL) and mitochondrial DNA copy number (mtDNAcn).

Telomeres are specialized nucleoprotein structures that “cap” the ends of linear chromosomes, effectively ensuring genomic stability.43–45 With increasing age and number of mitotic divisions, progressive TL shortening leads to replicative senescence, resulting in morphological and functional changes associated with loss of tissue function and disease.46 As such, TL shortening has been proposed to be a useful index of
biological aging. Importantly, TL shortening may be accelerated by oxidative stress and inflammation, which are also critical components underlying CVD. Previous studies, but not all, have reported significantly reduced TL in young and middle-aged/older adults who had experienced ACEs. Moreover, studies have largely indicated a significant inverse relationship between TL and central arterial stiffness. Together, these studies suggest that childhood adversity may impact cardiovascular health trajectories via accelerated biological and vascular aging. However, whether TL mediates the association between ACEs and central arterial stiffness has not yet been examined.

Similar to telomeres, mitochondria may represent a biological link between ACEs and cardiovascular health outcomes. Mitochondria house their own genome, which consists of a circular, double-stranded ≈16.6-kb mitochondrial DNA (mtDNA) that encodes for proteins essential to mitochondrial bioenergetics. MtDNA can exist in hundreds to thousands of copies per diploid cell, depending on the cell's energy demands. MtDNA is particularly susceptible to oxidative stress and inflammation. Thus, the extent of mtDNA damage and mitochondrial dysfunction may be indexed by mtDNAcn. Previous studies have documented an association between reduced mtDNAcn and the presence of CVD in humans and increased aortic stiffness in mice. Together, these findings suggest that mtDNAcn may be inversely associated with central arterial stiffness in humans; however, this has not yet been examined.

Recently, mtDNAcn has been postulated to mediate the relationship between ACEs and deleterious health outcomes. While 2 previous studies reported elevated mtDNAcn in individuals exposed to childhood adversity, mtDNAcn (as a marker of accelerated biological aging) is a potential mediator between ACEs and central arterial stiffness, attributable to chronic/repeated stress during childhood, we would hypothesize a lower mtDNAcn. Moreover, a lower mtDNAcn would be associated with greater central arterial stiffness (ie, faster cfPWV). This hypothesis is consistent with previous hypotheses suggesting that, following an initial adaptive increase in mtDNAcn, chronic/repeated biological stress may result in a reduction in mtDNAcn alongside mitochondrial dysfunction, which are key features of biological aging and critical to CVD pathology.

To summarize, our aim was to examine a model of accelerated biological aging as a novel pathway linking ACEs with greater central arterial stiffness—an intermediate cardiovascular health outcome associated with vascular aging and increased CVD risk—and to assess the potential mediating effects of TL and mtDNAcn on this association in a sample of healthy young adults. It was hypothesized that: (1) individuals who had experienced a greater number of ACEs would have a faster cfPWV; (2) individuals who had experienced a greater number of ACEs would present with evidence of accelerated biological aging, as indexed by either (a) shorter mean TL and/or (b) lower mean mtDNAcn; (3) shorter mean TL and/or lower mean mtDNAcn would be associated with a faster cfPWV; and (4) the association between ACEs and cfPWV would be mediated by either (a) mean TL and/or (b) mean mtDNAcn.

**METHODS**

**Study Population and Design**

The data that support the findings of this study are available from the corresponding author upon reasonable request. The current study was performed as part of the NLHS (Niagara Longitudinal Heart Study) in Niagara, ON, Canada. The NLHS is a follow-up study, building on 3 baseline studies that took place in the Niagara region from 2007 to 2012 (mean follow-up time 9.6±0.9 years; range, 8.6–12.2 years). As cfPWV, TL, and mtDNAcn were not assessed at baseline, only the follow-up sample was examined. At the time of analyses, 248 individuals had been reenrolled for follow-up assessment, with complete cardiovascular, biological, and ACEs data being available for 185 individuals. The NLHS protocol was approved by the university REB and all participants provided written informed consent.

**BP, Heart Rate, and Anthropometric Measures**

Participants were tested at the Human Hemodynamics Laboratory at Brock University. All hemodynamic data were collected in a quiet, dimly lit, and temperature-controlled room under standardized conditions. Briefly, participants were instructed to abstain from vigorous physical activity (PA) and alcohol and caffeine consumption for at least 12 hours, and to fast for at least 4 hours before testing. Upon arrival, participants were asked to void their bladder to prevent any effect of bladder distension on arterial BP. Standing height (cm) was measured using a stadiometer (STAT-7X, Ellard Instrumentation, Monroe, WA) and body mass (kg) was measured using a digital scale (BWB-800S, Tanito, Tokyo, Japan). Body mass index (BMI; kg/m²) was calculated as body mass (kg) divided by height squared (m²) and was then categorized as normal weight (<25 kg/m²), overweight (25–29.9 kg/m²), or obese (≥30 kg/m²).

**Assessment of Beat-by-Beat BP and Central Arterial Stiffness**

Following anthropometric measures, participants assumed a supine position where they remained...
for 10 minutes of quiet rest and were instrumented. Continuous heart rate (HR) was assessed using a standard single-lead ECG, and beat-by-beat BP was assessed noninvasively at the left middle finger via finger photoplethysmography (Nexfin, BMYEYE, Amsterdam, Horton, Norway; NIBP Nano, ADInstruments, Colorado Springs, CO). Beat-by-beat BP was calibrated to the average of 3 manual BP measurements taken simultaneously at the right brachial artery (sphygomanometry). Continuous HR and beat-by-beat BP data were collected in real-time throughout the duration of testing using a commercially available data acquisition system (PowerLab; ADInstruments, Colorado Springs, CO) and analyzed offline using the system’s accompanying software (LabChart 8; ADInstruments). One-minute averages of brachial-adjusted beat-by-beat systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure [mean arterial pressure = DBP + 1/3(SBP − DBP)], as well as HR were calculated from the last minute of baseline supine data collection.

Measurement of cfPWV was used to assess central arterial stiffness and was defined as the speed of the BP (ie, pulse) waveform from the left CCA to the left femoral artery. To obtain cfPWV, local pulse pressure waveforms were collected at the CCA and femoral artery, and 15 of the most well-defined and consistent waveforms were averaged in the calculation of pulse transit time. Values for cfPWV are expressed in m/s.

Assessment of Absolute TL and mtDNAcn
Absolute TL and mtDNAcn were quantified using genomic DNA isolated from saliva in the Inflammation & Immunity Laboratory at Brock University. Participants were asked to provide 2 mL of liquid saliva (ie, bubbleless) into saliva collection tubes (Oragene-DNA, DNA Genotek, #OG-500, Ottawa, ON, Canada). Samples were stored at room temperature before DNA isolation. Nuclear and mtDNA were isolated from the samples using commercially available DNeasy Blood and Tissue Kits (QIAGEN Inc., #69506, Toronto, ON, Canada) per the manufacturer’s protocol. DNA purity was assessed by determining the absorbance ratio of DNA in eluate at 260 and 280 nm wavelength light via spectrophotometry (NanoVue Plus Spectrophotometer, GE Healthcare Life Sciences, Mississauga, ON, Canada), with absorbance ratios of 1.8 to 2.0 being considered acceptable purity. Samples were stored at −20 °C until further analyses.

Absolute TL and mtDNAcn were quantified using a commercially available quantitative polymerase chain reaction (qPCR) assay kit (Absolute Human Telomere Length and Mitochondrial DNA Copy Number Dual Quantification qPCR Assay Kit, ScienCell Research Laboratories, #8958, Carlsbad, CA). For proprietary reasons, the sequences of the forward and reverse primers for both the telomeric and mitochondrial sequences, and the single copy reference gene, were unavailable to the researchers. Primer sets used in the current study were validated by qPCR with melt-curve analysis and gel electrophoresis for amplification specificity, and by template serial dilution for amplification efficiency.

Primer sets for each amplicon were added to nuclease-free water and KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems Inc., Wilmington, MA) in a ratio of 1:3:5:5. Genomic samples (both participant and reference samples) were loaded in triplicate on a 96-well qPCR plate and a negative control was loaded in duplicate. The qPCR was conducted in a StepOnePlus Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific, Waltham, MA) with a thermocycling profile as follows: 32 cycles of initial denaturation at 95 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 45 s. Immediately following qPCR, melt-curve analysis was performed to ensure primer specificity. Mean relative TL and mean relative mtDNAcn were calculated according to the formula by Cawthon (2002): $2^{-\Delta \Delta C_t}$, where $\Delta C_t$ is the value of the comparative threshold cycle analysis for either TL or mtDNAcn relative to the single copy reference gene. Absolute TL and mtDNAcn were then quantified as the product of the reference sample values of known TL and mtDNAcn by the relative values of each target sample (eg, reference sample TL × $2^{-\Delta \Delta C_t(TL)}$ of participant sample).

Assessment of Serum C-Reactive Protein Concentration
Serum C-reactive protein (CRP) concentrations (mg/L) were measured in the Inflammation & Immunity Laboratory and used as a marker of inflammation, as subclinical inflammation has been associated with arterial stiffness, and both TL and mtDNAcn. Venous blood (15 mL) was drawn from the antecubital fossa of each participant by a licensed nurse. Serum was separated from 5 mL of blood by centrifugation (1500g at 4 °C for 10 minutes) and stored at −80 °C. A commercially available ELISA (R&D Systems, #DCRP00, Minneapolis, MN) was used for the quantification of serum CRP concentrations.
Assessment of Childhood Adversity and Health Behaviors

ACEs were assessed (pre-COVID-19) using the Childhood Trust Events Survey 2.0—a 26-item inventory adapted from the Traumatic Stress Survey that screens for exposure to traumatic childhood events occurring before the age of 18 years. For comparability, we included 13 items of the Childhood Trust Events Survey 2.0 that mirrored the 8 ACE domains identified in the original ACE study. These items focused on experiencing childhood maltreatment, including sexual (2 items), physical (1 item), and emotional abuse (1 item), and severe household dysfunction, including witnessing domestic violence (2 items), having someone in the household suffering from serious mental illness or suicidal ideation (2 items), neglect attributable to a family member being addicted to drugs or alcohol (2 items) or being incarcerated (1 item), and an unexpected death or separation from a family member, including divorce or separation (2 items). Previous studies have provided support for the retrospective self-reporting of ACEs in adulthood with 1 study identifying good test–retest reliability (Cohen kappa values of 0.6–0.7 over 1 year) when assessing childhood maltreatment. Thus, findings suggest that individuals are willing to report ACEs when they are in a safe, comfortable environment.

In accordance with the original ACE study and our groups’ previous work, positive response for any item was coded as a positive response for the ACE domain, which were then summed to create a scale ranging from 0 to a threshold value of ≥4 ACEs.

Information on smoking status, PA level, and perceived recent life stress were also assessed. Smoking status was determined by asking the question “Do you currently smoke cigarettes daily, occasionally, or not at all?” and was then categorized into 3 groups: regular smokers (smoked ≥1 cigarette per day), occasional smokers (smoked <1 cigarette per day), and nonsmokers (did not smoke cigarettes). PA level was assessed using the International Physical Activity Questionnaire Short Form and was categorized as low active (<600 metabolic equivalent-minutes per week), moderate active (600–2999 metabolic equivalent-minutes per week), and high active (≥3000 metabolic equivalent-minutes per week). Perceived recent life stress was measured using the Perceived Stress Scale, which consists of 14 items that assess the extent to which events are appraised as being stressful. A 5-point Likert-type scale ranging from 0 (“Never”) to 4 (“Very likely”) was used to score each item. Higher scores on the Perceived Stress Scale indicate greater perceived recent life stress.

Statistical Analysis

Continuous variables are presented as the mean±SE, and categorical variables are presented as absolute numbers and relative percentages. Shapiro–Wilks tests of normality assessed the distribution of values for continuous variables. Data pertaining to the distribution of TL, mtDNAcn, and serum CRP concentration were significantly skewed and were log-transformed to better approximate a normal distribution. Skewness coefficients for TL, mtDNAcn, and CRP were 2.36, 2.32, and 3.07, respectively, before log-transformation, and 0.67, 0.21, and 0.20, respectively, following log-transformation. Log-transformed values were used in all analyses and are presented in the Results section. Pearson correlation coefficients were calculated to assess bivariate associations between TL, mtDNAcn, and cfPWV. Multiple linear regression analyses were used to examine the associations between ACEs, TL, mtDNAcn, and cfPWV, adjusting for relevant covariates determined a priori: age, sex, smoking status, BMI, PA, HR, SBP, and serum CRP. Covariates were selected based on previous research demonstrating their importance in predicting cfPWV.

RESULTS

Participant characteristics are presented in Table 1. One hundred and eighty-five participants (n=102 women) were included in the current analyses. Mean cfPWV was 5.8±0.1 m/s. Mean absolute TL and mtDNAcn were 649.9±58.8 kb per diploid cell and 624.0±34.5 copies per diploid cell, respectively. Mean log(TL) and log(mtDNAcn) were 2.6±0.03 kb per diploid cell and 2.7±0.02 copies per diploid cell, respectively. The mean number of ACEs reported in the current sample was...
**ACEs, TL, mtDNAcn, and Arterial Stiffness**

The bivariate correlation between ACEs and cfPWV was significant ($r=0.201$, $P=0.006$). ACEs remained a significant positive predictor of cfPWV ($\beta=0.147$, $P=0.035$) after adjusting for age, sex, smoking status, BMI, PA, HR, SBP, and serum CRP (Table 2). Age, regular smoking, BMI, PA, SBP, and HR were also significant predictors of cfPWV in this model (all $P<0.05$).

**Association Among ACEs, TL, and mtDNAcn**

ACEs were not significantly correlated with log(TL) ($r=-0.014$, $P=0.846$) or log(mtDNAcn) ($r=0.017$, $P=0.820$). In contrast, log(TL) and log(mtDNAcn) were significantly correlated ($r=0.558$, $P=0.001$) (Figure 1). Consistent with these nonsignificant correlations, ACEs remained a non-significant predictor of both log(TL) ($\beta=-0.027$, $P=0.726$) and log(mtDNAcn) ($\beta=-0.038$, $P=0.620$) after adjusting for relevant covariates in multiple linear regression analysis (Table 3; Model 1 and Model 2, respectively). Moreover, all covariates remained nonsignificant with the exception of regular smoking on log(mtDNAcn). Perceived recent life stress was not associated with either log(TL) or log(mtDNAcn), and the addition of this covariate to subsequent regression models had negligible effects on overall model fit (data not shown).

**Association Among TL, mtDNAcn, and cfPWV**

cfPWV was significantly inversely associated with log(TL) ($r=-0.176$, $P=0.016$) but was not associated with log(mtDNAcn) ($r=-0.142$, $P=0.054$) (Figure 2). However, after adjusting for covariates in multiple linear regression analysis, both log(TL) and log(mtDNAcn) were significant predictors of cfPWV (Table 4; Model 1 and Model 2, respectively). In Model 1, log(TL) was a significant negative predictor of cfPWV ($\beta=-0.170$, $P=0.011$). In Model 2, log(mtDNAcn) was a significant negative predictor of cfPWV ($\beta=-0.159$, $P=0.019$). Additionally, in both models, age, regular smoking, BMI, SBP, and HR were significant predictors of cfPWV (all $P<0.05$).

**Mediation Analyses of TL and mtDNAcn on the Association Between ACEs and cfPWV**

The potential mediating effects of log(TL) and log(mtDNAcn) on the association between ACEs and cfPWV were assessed. Based on the above multiple
linear regression models (Table 3), no mediation effect was anticipated for either log(TL) or log(mtDNAcn). In a regression model that included both ACEs and log(TL), both were significant but independent predictors of cfPWV (Figure 3A; model not shown). These results were the same in a regression model that examined both ACEs and log(mtDNAcn) (Figure 3B; model not shown). The standardized regression coefficients obtained from the regression models including both ACEs and mediating variables can be observed in round brackets in Figure 3A and 3B, respectively.

DISCUSSION

This study examined the relationship between ACEs and central arterial stiffness (cfPWV) and assessed the potential mediating effects of markers of biological aging (ie, TL and mtDNAcn) on this association in a sample of healthy young adults. As hypothesized, a greater number of ACEs were significantly associated with faster cfPWV, independent of age, sex, smoking status, BMI, PA, HR, SBP, and serum CRP. Furthermore, lower TL and mtDNAcn were both significantly associated with a faster cfPWV in models adjusted for covariates. Finally, neither TL nor mtDNAcn were associated with ACEs, indicating that neither marker mediated the association between ACEs and cfPWV, but were significant, independent predictors.

CVD risk begins to develop throughout childhood and adolescence, culminating in disease later in life via several traditional risk factors. Additionally, ACEs may contribute to increased CVD risk through their impact on the structure and function of central arteries. Previous studies have revealed associations between ACEs and parameters of deleterious cardiovascular remodeling, including increased arterial stiffness, which is a key component of biological and vascular aging and CVD pathology. For example, a recent study found that individuals exposed to >4 ACEs had a greater increase in ECG-toe PWV compared with those exposed to <4 ACEs over a 9-year period from childhood, independent of sex and changes in SBP, HR, BMI, and PA. Similarly, Su et al found that healthy adolescents and young adults (21.3±2.8 years; n=221) exposed to ≥2 ACEs had a faster CCA-radial PWV compared with those exposed to <2 ACEs, and Bomhof-Roordink et al found a significant positive
association between childhood trauma and central augmentation index in a population of middle-aged adults (46.5±12.1 years; n=650). Our findings provide further evidence of a susceptibility of the arterial vasculature to undergo wall stiffening in response to ACEs, thereby supporting the hypothesis that ACEs may impact cardiovascular health via accelerated biological aging.

In the context of accelerated biological aging, ACEs are thought to “get under the skin” and influence cardiovascular health, in part, via dysregulation of the stress response systems (ie, the hypothalamic–pituitary–adrenal axis, sympathetic nervous system, and immune system). Maladaptive metabolic and immune responses have been implicated as potential mediators underpinning the ACEs-cardiovascular health relationship. Specifically, it has been postulated that childhood adversity disrupts allostasis, leading to physiological dysfunction across the stress response systems and ultimately resulting in elevated biological stress (ie, oxidative and inflammatory stress) via downstream metabolic and inflammatory pathways. Importantly, elevated biological stress is thought to promote biological and vascular aging, which, as previously described, is associated with increased central arterial stiffness and CVD risk. Moreover, this hypothesis lends itself to the biological embedding model of early adversity, which maintains that childhood stress gets “programmed” into immune cells, resulting in proinflammatory tendencies that may drive CVD pathogenesis. Here, 2 indices of biological aging were examined as potential mediators of the association between ACEs and central arterial stiffness—TL and mtDNAcn.

Several previous studies have examined the association between ACEs and TL, whereas few have examined the association between ACEs and mtDNAcn and none have examined these markers as potential mediators of the association between ACEs and cardiovascular health outcomes. Interestingly, neither TL nor mtDNAcn was associated with ACEs in the current study, which contradicted the findings of several previous reports but were in agreement with several others. One potential reason for the findings of a null association between ACEs and TL in the current study, as well as the significant heterogeneity across study findings, may be that TL does not provide a sensitive measure of ACE-associated biological stress, particularly in young adulthood. Indeed, meta-analyses that have aimed to elucidate

**Table 3. Multiple Linear Regression Main Effect of ACEs and Covariates on Log(TL) (Model 1) and Log(mtDNAcn) (Model 2) (n=185)**

| Model | Variables | B(SE) | β | 95% CI LL | UL | P value |
|-------|-----------|-------|---|----------|----|---------|
| Model 1 | Age, y | −0.015 (0.021) | −0.056 | −0.057 | 0.027 | 0.471 |
|       | Sex† | 0.071 (0.066) | 0.088 | −0.059 | 0.201 | 0.284 |
|       | Occ. Smoke‡ | −0.149 (0.125) | −0.092 | −0.396 | 0.097 | 0.233 |
|       | Reg. Smoke‡ | 0.118 (0.130) | 0.075 | −0.139 | 0.374 | 0.367 |
|       | BMI | 0.003 (0.006) | 0.037 | −0.010 | 0.015 | 0.663 |
|       | PA | 0.018 (0.031) | 0.046 | −0.043 | 0.078 | 0.569 |
|       | Log(CRP) | −0.020 (0.065) | −0.028 | −0.147 | 0.108 | 0.760 |
|       | ACEs | −0.008 (0.022) | −0.027 | −0.050 | 0.035 | 0.726 |
| Model 2 | Age, y | −0.017 (0.014) | −0.090 | −0.046 | 0.011 | 0.233 |
|       | Sex† | 0.019 (0.045) | 0.034 | −0.069 | 0.107 | 0.676 |
|       | Occ. Smoke‡ | −0.010 (0.084) | −0.009 | −0.177 | 0.156 | 0.903 |
|       | Reg. Smoke‡ | −0.227 (0.088) | −0.210 | 0.054 | 0.401 | 0.011 |
|       | BMI | 0.001 (0.004) | 0.004 | −0.008 | 0.009 | 0.963 |
|       | PA | 0.029 (0.021) | 0.110 | −0.012 | 0.070 | 0.160 |
|       | Log(CRP) | −0.005 (0.044) | −0.010 | −0.091 | 0.081 | 0.908 |
|       | ACEs | −0.007 (0.015) | −0.038 | −0.036 | 0.022 | 0.620 |

ACEs indicates adverse childhood experiences (0, 1, 2, 3, or ≥ 4); B, the unstandardized regression coefficient; BMI, body mass index (kg/m²); LL, lower limit of the 95% CI; Log(CRP), log-transformed serum C-reactive protein concentration (mg/L); Occ. Smoke, occasional smoking; PA, physical activity (metabolic equivalent-minutes/week); Reg. Smoke, regular smoking; TL, upper limit of the 95% CI; and β, standardized regression coefficient. P<0.05 indicates significance.

*Cohen F of Model 1=0.029, P=0.754; R² of Model 1=0.028. Cohen F of Model 2=0.166, P=0.066; R² of Model 2=0.142.
†Reference group for sex is men.
‡Reference group for smoking status is nonsmokers.
§P<0.05.
the relationship between childhood adversity and TL have reported an overall negative association with aggregated small effect sizes,\textsuperscript{105–107} or no association at all.\textsuperscript{114} Moreover, these analyses have consistently reported significant heterogeneity across study findings,\textsuperscript{105–107} which may, in part, be attributable to differences in study approaches, sample sizes, age of participants, measures of childhood adversity, and/or method of TL measurement.\textsuperscript{23,54}

Notably, the regulation of TL is considered a highly dynamic process with several eroding and protective factors.\textsuperscript{95,115} These factors may modulate any association between ACEs and TL, such that reported findings are influenced by some unknown variable(s). Various psychological (eg, positive effect and resilience)\textsuperscript{116–118} and biological factors (eg, telomerase activity)\textsuperscript{45,119} have been shown to influence TL, and these factors may modulate the association between ACEs and TL by directly influencing biological processes critical to telomere lengthening or indirectly by reducing an individual’s susceptibility to the attritional effects of stress.\textsuperscript{117} Additionally, how an ACE is perceived (ie, the level of stress or allostatic load associated with a given ACE) may also influence the purported association between ACEs and TL.\textsuperscript{38} Indeed, a significant, albeit small, negative association between perceived stress and TL has been reported in the literature,\textsuperscript{120} supporting the notion of a subjective component to the association between ACEs and TL. Accordingly, ACEs may be associated with reduced TL in those who perceive their childhoods as being stressful and still “wear” the associated allostatic load.

Finally, the presence of recent life stress may also influence any association between ACEs and TL.\textsuperscript{103} Both Verhoeven et al and van Ockenburg et al found recent stressful life events (ie, within the past year), but not childhood adversity, to be independently associated with reduced TL in large cohorts (n=2936 and n=1094, respectively) of healthy middle-aged adults (41.8±13.1 years and 53.1±11.4 years, respectively).\textsuperscript{56,57} Further, Willis et al found that stressful life events in adulthood fully mediated the association between childhood adversity and TL in a large sample of healthy older adults (69.3±10.3 years; n=5754).\textsuperscript{103} Together, these studies suggest that any deleterious effects of ACEs on TL may be more marked with a shorter temporal proximity between adversity exposure and TL measurement.\textsuperscript{23,103,105,106}

Interestingly, however, perceived recent life stress was not associated with TL (or mtDNAcn) in the current study, and its addition to models predicting TL had negligible effects on overall model fit (data not shown). Alternatively, any effect of ACEs on TL that may present in middle-age/older adulthood\textsuperscript{24,25} might result from a reduced ability to cope with adversity\textsuperscript{106,116–118} and/or the adoption of negative coping strategies (eg, smoking, alcohol misuse).\textsuperscript{13,14,22,105} However, ascertaining this would require longer-term follow-up data (eg, 20 years versus ≈10 years), which were not available for this study.

Similar to TL, mtDNAcn was not associated with ACEs in the current study, which contradicted the findings of 2 previous reports.\textsuperscript{59,70} As previously described, a key difference between the current study and previous studies was that the current study aimed, in part, to examine mtDNAcn as a potential mediator of the association between ACEs and central arterial stiffness. Consistent with previous hypotheses on mtDNAcn dynamics,\textsuperscript{58} it was postulated that an accumulation of ACEs (up to ≥4 ACEs) would indicate chronic/repeated stress during childhood, which, in turn, would be associated with elevated biological stress (ie, oxidative stress\textsuperscript{37,121} and inflammation\textsuperscript{25,122,123}) and accelerated biological aging, as indexed by a lower mtDNAcn.\textsuperscript{68} Moreover, a lower mtDNAcn would be associated with greater central arterial stiffness (ie, faster cfPWV), thereby mediating, in part, the association between ACEs and central arterial stiffness, and providing support for the hypothesis that ACEs may accelerate biological/vascular aging.\textsuperscript{13} Regardless, the current study

---

**Figure 2.** Association between carotid-femoral pulse wave velocity and 2 markers of biological aging: telomere length and mitochondrial DNA copy number.

**A.** Correlation of carotid-femoral pulse wave velocity (m/s) with log-transformed telomere length; kilobases per diploid cell.  
**B.** Correlation of carotid-femoral pulse wave velocity with log-transformed mitochondrial DNA copy number (copies per diploid cell). cfPWV indicates carotid-femoral pulse wave velocity; Log(TL), log-transformed absolute telomere length; and Log(mtDNAcn), log-transformed absolute mitochondrial DNA copy number.

---

\[ R = 0.176 \]
\[ R^2 = 0.031 \]
\[ P = 0.016 \]

\[ R = 0.142 \]
\[ R^2 = 0.020 \]
\[ P = 0.054 \]

---

\[ R^2 = 0.054 \]
\[ R^2 = 0.142 \]
found no association between ACEs and mtDNAcn. The exact reasons for these discrepant findings are unclear; however, they may be because of differences in study approach, age of participants, measures of ACEs, or method of mtDNAcn measurement.

Tyrka et al first reported that mtDNAcn was significantly higher among healthy adults (31.0±10.7 years; n=290) exposed to moderate–severe childhood maltreatment.59 The authors speculated that in healthy adults, increased mtDNAcn may be an early compensatory response that could be limited by time and disease burden.59 One major methodological difference between the current study and that of Tyrka et al was the source tissue used for mtDNAcn measurement. Indeed, Tyrka et al used whole blood for the measurement of mtDNAcn, whereas the current study used saliva. Although both source tissues contain leukocytes, whole blood additionally contains platelets, and saliva additionally contains buccal epithelial cells. To date, no study has compared saliva mtDNAcn with that of blood and thus, it remains unclear whether saliva mtDNAcn is representative of mtDNAcn in other tissues. Previous reports using whole blood samples have revealed a significant influence of platelet and leukocyte count on mtDNAcn, such that mtDNAcn is positively associated with platelet count and inversely associated with leukocyte count.124 However, the current lack of studies examining mtDNAcn dynamics in various tissues makes it difficult to compare the findings of the current study with those of Tyrka et al.

In another study, Ridout et al found mtDNAcn to be significantly higher in preschool-aged children (4.3±0.7 years; n=256) with substantiated cases of childhood maltreatment compared with nonexposed children.70 Two key differences in study methodologies may, in part, explain the differences in study findings. First, mtDNAcn was measured within 1 year post-ACE exposure in the study by Ridout et al, whereas mtDNAcn was measured up to 25 years (and at minimum

| Model* | Variables | B(SE) | β  | 95% CI | P value |
|--------|-----------|-------|-----|--------|--------|
|        |           |       |     | LL     | UL     |
| Model 1 | Age, y    | 0.097 (0.041) | 0.158 | 0.015  | 0.179  | 0.020§ |
|        | Sex†      | 0.047 (0.139) | 0.026 | −0.228 | 0.321  | 0.738  |
|        | Occ. Smoke‡ | 0.418 (0.245) | 0.116 | −0.065 | 0.901  | 0.089  |
|        | Reg. Smoke‡ | 0.664 (0.253) | 0.191 | 0.164  | 1.163  | 0.010§ |
|        | BMI       | 0.034 (0.013) | 0.206 | 0.008  | 0.059  | 0.009§ |
|        | PA        | −0.105 (0.059) | −0.123 | −0.222 | 0.012  | 0.078  |
|        | SBP       | 0.015 (0.006) | 0.184 | 0.003  | 0.028  | 0.018  |
|        | HR        | 0.016 (0.006) | 0.182 | 0.024  | 0.039  | 0.010§ |
|        | Log(CRP)  | 0.034 (0.128) | 0.021 | −0.219 | 0.286  | 0.793  |
|        | Log(TL)   | −0.376 (0.148) | −0.170 | −0.664 | −0.087 | 0.011§ |
| Model 2 | Age, y    | 0.094 (0.042) | 0.153 | 0.012  | 0.176  | 0.025§ |
|        | Sex†      | 0.030 (0.139) | 0.017 | −0.245 | 0.305  | 0.828  |
|        | Occ. Smoke‡ | 0.474 (0.244) | 0.132 | −0.008 | 0.956  | 0.054  |
|        | Reg. Smoke‡ | 0.680 (0.258) | 0.196 | 0.171  | 1.189  | 0.009§ |
|        | BMI       | 0.033 (0.013) | 0.199 | 0.007  | 0.059  | 0.012§ |
|        | PA        | −0.097 (0.060) | −0.114 | −0.215 | 0.020  | 0.104  |
|        | SBP       | 0.015 (0.006) | 0.184 | 0.003  | 0.028  | 0.018§ |
|        | HR        | 0.017 (0.006) | 0.194 | 0.005  | 0.030  | 0.006§ |
|        | Log(CRP)  | 0.033 (0.128) | 0.021 | −0.220 | 0.286  | 0.797  |
|        | Log(mtDNAcn) | −0.511 (0.216) | −0.159 | −0.934 | −0.084 | 0.019§ |

*B indicates the unstandardized regression coefficient; BMI, body mass index (kg/m²); HR, 1-minute average of continuous heart rate (bpm); LL, lower limit of the 95% CI; Log(CRP), log-transformed serum C-reactive protein concentration (mg/L); Log(mtDNAcn), log-transformed absolute mitochondrial DNA copy number; Occ. Smoke, occasional smoking; PA, physical activity (metabolic equivalent-minutes/week); Reg. Smoke, regular smoking; SBP, 1-minute average of beat-by-beat systolic blood pressure (mm Hg); Log(TL), log-transformed absolute telomere length; UL, upper limit of the 95% CI; and β, standardized regression coefficient. P<0.05 indicates significance.

*Cohen f² of Model 1=0.362, P<0.001; R² of Model 1=0.266. Cohen f² of Model 2=0.355, P<0.001; R² of Model 2=0.262.

†Reference group for sex is men.
‡Reference group for smoking status is nonsmokers.
§P<0.05.
Figure 3. Mediation analyses of telomere length and mitochondrial DNA copy number on the association between ACEs and carotid-femoral pulse wave velocity.

A. Mediation model examining the mediating effect of log-transformed telomere length; kilobases per diploid cell on the association between ACEs and carotid-femoral pulse wave velocity (m/s). B. Mediation model examining the mediating effect of log-transformed mitochondrial DNA copy number; copies per diploid cell on the association between ACEs and carotid-femoral pulse wave velocity. Values in parentheses are standardized regression coefficients obtained from regression models including ACEs and log-transformed telomere length and ACEs and log-transformed mitochondrial DNA copy number, respectively, as predictors of carotid-femoral pulse wave velocity. ACEs indicates adverse childhood experiences; cfPWV, carotid-femoral pulse wave velocity; Log(mtDNAcn), log-transformed absolute mitochondrial DNA copy number; Log(TL), log-transformed absolute telomere length; and β, the standardized regression coefficient. P<0.05 indicates significance.

1 year) post-ACE exposure in the current study. Similar to TL, it may be that recent adversity is more closely associated with alterations in mtDNAcn and that with the passage of time, mtDNAcn may recover. Thus, with respect to the current study, the temporal proximity of ACEs to mtDNAcn measurement may have nullified any impact that ACEs may have had on mtDNAcn. Second, Ridout et al examined the association between ACEs and mtDNAcn in preschool-aged children, whereas the current study examined this association in young adults. Preschool-age represents a period of significant growth, and it would be unsurprising to observe a rapid increase in mtDNAcn (with increased mitochondrial biogenesis) during this period because of the high rate of cellular proliferation. In contrast, mtDNAcn remains relatively stable throughout young- and middle-adulthood and slightly declines after the age of 50 years. The regulation of mtDNAcn is a dynamic process influenced by mitochondrial biogenesis, which, in itself, is influenced by age and various other endogenous and environmental factors. Thus, age is a likely modulator of the purported association between ACEs and mtDNAcn, and this may partly explain differences across study findings. Similar to TL, any effect of ACEs on mtDNAcn that may present in middle-age/older adulthood might result from a reduced ability to cope with adversity and/or the adoption of negative coping strategies; however, again, this would require longer-term follow-up data, which were not available for the current study.

The null association between ACEs and both TL and mtDNAcn in the current study suggest that these markers do not provide an adequate index of ACE-associated biological stress/aging in healthy young adults. Moreover, the null association between ACEs and these markers preclude any mediational effect that they may have had on the association between ACEs and cfPWV. Accordingly, TL and mtDNAcn likely do not represent a biological link between ACEs and central arterial stiffness. Interestingly, however, the current study found that both TL and mtDNAcn predicted cfPWV, independent of ACEs and other traditional CVD risk factors. These findings agreed with several previous reports (but not all) that have examined TL and mtDNAcn in the context of central arterial stiffness. Additionally, and consistent with previous reports, the current study found that TL and mtDNAcn were positively correlated. Previous studies have linked telomeres and mitochondria at a functional level, and the “telomere-mitochondria axis” has been postulated to contribute to CVD pathology. Accordingly, reductions in these markers may represent mechanistic factors contributing to central arterial stiffness or epiphenomena of the biological/vascular aging process. Future studies are required to better elucidate the relationship between TL, mtDNAcn, and central arterial stiffness.

Strengths and Limitations

First, to our knowledge, this was the first study to examine a model of accelerated biological aging (ie, reduced TL and/or lower mtDNAcn) as a novel pathway linking ACEs to increased central arterial stiffness (ie, faster cfPWV). Although the current study did not find a significant mediating effect of either TL or mtDNAcn on the association between ACEs and cfPWV, both TL and mtDNAcn, along with ACEs, independently predicted cfPWV. However, we were unable to examine
changes over time because of the single point of data collection of TL, mtDNAcn, and cfPWV. Future experimental and longitudinal studies may aim to better elucidate the relationship between ACEs, TL, and mtDNAcn, and central arterial stiffness, as well as the factors (eg, biological and behavioral factors) that may influence these relationships.

Second, self-report questionnaires for the assessment of ACEs are subject to both self-report and recall bias. However, the inclusion of young adults (aged 19–25 years) may reduce recall bias because of a shorter temporal proximity between exposure and reporting. Additionally, the Childhood Trust Events Survey 2.0 was administered at the end of the laboratory visit and near the end of a broader questionnaire package. At this time, the researchers had built a rapport with the participant and, ideally, created an optimal environment for the administration of the questionnaire. It has been reported that when conducted in a dignified manner, there is a high level of compliance, reliability, and accuracy in the reporting of ACEs.88,90 Finally, although childhood adversity can be assessed via interview,135 a self-report questionnaire was used in the current study as a means of minimizing participant discomfort and social response bias.88,89

Third, the current study used saliva as a source tissue for the assessment of TL and mtDNAcn. A potential concern about the use of saliva for TL measurement is the presence of buccal epithelial cells, as TL from this cell type does not show the same negative association with age that TL from other source tissues (eg, blood) have shown.103,136 However, several studies have demonstrated a significant correlation between TL extracted from saliva and TL extracted from blood, and concluded that although TL measurements from different source tissues are difficult to compare directly, the lower cost and less invasive nature of obtaining saliva samples makes them a reasonable measure of TL.136–138 It should also be noted that although both saliva and blood samples contain leukocytes, blood samples (specifically, whole blood samples) additionally contain platelets, which can influence mtDNAcn.124,125 To date, no study has compared saliva mtDNAcn with blood mtDNAcn and thus, it remains unclear if saliva mtDNAcn is representative of mtDNAcn in other tissues. However, because of the influence of source tissue (and cellular composition124,125), the null results here may be inherently limited in their generalizability. Future studies may aim to replicate the findings by using other source tissues for the measurement of TL and mtDNAcn.

CONCLUSIONS

To our knowledge, the current study demonstrated for the first time that an increasing number of ACEs were associated with a faster cfPWV, indicating a greater degree of central arterial stiffness. It was also found that ACEs were not associated with either TL or mtDNAcn, and neither biological marker mediated the association between ACEs and cfPWV. Based on these findings, it was concluded that TL and mtDNAcn likely do not represent a mechanistic pathway linking childhood adversity to central arterial stiffness, at least among young adults. Future studies may aim to examine alternative pathways linking childhood adversity and arterial stiffness, such as behavioral or mental health pathways, or alternative biological pathways to better understand how ACEs “get under the skin” and influence cardiovascular health. The current study also demonstrated a significant negative association between both TL and mtDNAcn with cfPWV. Reductions in these markers may represent mechanistic factors contributing to central arterial stiffness as well as epiphenomena of the biological/vascular aging process. Future studies are required to better elucidate the relationship between TL, mtDNAcn, and central arterial stiffness.

ARTICLE INFORMATION

Received April 29, 2022; accepted September 22, 2022.

Affiliations

Department of Health Sciences, Faculty of Applied Health Sciences (N.J.I., T.J.W., K.S.D., J.M., A.J.M., D.D.O.) and Brock-Niagara Centre for Health and Well-Being, Brock University, St. Catharines, Ontario, Canada (N.J.I., T.J.W., K.S.D., D.D.O.).

Acknowledgments

The authors acknowledge previous graduate students Aindriu Maguire, Kingston Wong, and Megan Henry for their work on the Niagara Longitudinal Heart Study (NLHS) project, as well as all the NLHS participants. Data were collected and analyzed at Brock University (Human Hemodynamics Laboratory and Inflammation & Immunity Laboratory). Dr. Wadeand Dr. O’Leary are principle investigators for the NLHS on which this analysis was based. Cardiovascular data were collected and analyzed by Dr. Dempster and N. Iannarelli. qPCR analyses were performed by J. Moore and N. Iannarelli under the direction of Dr. Moore and N. Iannarelli under the direction of Dr. MacNeil. N. Iannarelli, Dr. MacNeil, Dr. O’Leary, and Dr. Wade were responsible for the conceptualization and data analysis of the current work, with N. Iannarelli drafting the original manuscript. All authors were involved in interpretation of the data and revising the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Sources of Funding

The Niagara Longitudinal Heart Study is funded by the Canadian Institute of Health Research (CIHR #363174 and #399332). Dr. Dempster was funded by a CIHR Doctoral Research Award—Frederick Banting and Charles Best Canada Graduate Scholarship (RFN #167014).

Disclosures

None.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton A2, Benjamin EJ. Global burden of cardiovascular diseases and risk factors, 1990–2019. J Am Coll Cardiol. 2020;76:2982–3021. doi: 10.1016/j.jacc.2020.11.010

2. Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors. J Am Coll Cardiol. 2019;74:2529–2532. doi: 10.1016/j.jacc.2019.10.009
a systematic review and meta-analysis. Lancet Public Health. 2019;4:e517–e528. doi: 10.1016/S2468-2667(19)30145-8

21. Hughes K, Bellis MA, Hardcastle KA, Sethi D, Butchart A, Miktion C, Jones L, Dunne MP. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Health. 2017;2:e356–e366. doi: 10.1016/S2468-2667(17)30118-4

22. Su S, Jimenez MP, Roberts CTF, Loucks EB. The role of adverse childhood experiences in cardiovascular disease risk: a review with emphasis on plausibility mechanisms. Curr Cardiol Rep. 2015;17:88. doi: 10.1007/s11886-015-0645-1

23. Bürgin D, O’Donovan A, d’Huart D, di Gallo A, Eckert A, Feger J, Schneck K, Schmid M, Booman M. Adverse childhood experiences and telomere length: a look into the heterogeneity of findings—a narrative review. Front Neurosci. 2019;13:490. doi: 10.3389/fnins.2019.00490

24. Puterman E, Gemmill A, Karasek D, Weir D, Adler NE, Prather AA, Epel ES. Lifespan adversity and later adulthood telomere length in the nationally representative US health and retirement study. Proc Natl Acad Sci U S A. 2016;113:6335–6342. doi: 10.1073/pnas.1525602113

25. Kiec-Glaser JK, Gouin J-F, Weng M, Malarkey WB, Beversdorf DQ, Glaser R. Childhood adversity heightens the impact of later-life caring stress on telomere length and inflammation. Psychosom Med. 2011;73:16–22. doi: 10.1097/PSY.0b013e31820573b6

26. Lona G, Hauser C, Köchli S, Infanger D, Endes K, Schmitt-Trucksass A, Hanssen A. Association of blood pressure, obesity and physical activity with arterial stiffness in children: a systematic review and meta-analysis. Pediatr Res. 2021;91:502–512. doi: 10.1038/s41390-021-01278-5

27. Bomhoff-Roorink H, Seldenhin A, van Hout HPJ, van Marwick HWJ, Diamant M, Penninx BWJH. Associations between life stress and subclinical cardiovascular disease are partly mediated by depressive and anxiety symptoms. J Psychosom Res. 2015;78:332–339. doi: 10.1016/j.jpsychores.2015.02.009

28. Klassen SA, Chinico D, O’Leary DD, Carney M, Wade TJ. Linking systemic arterial stiffness in adolescents to adverse childhood experiences. Child Abuse Negl. 2016;56:1–10. doi: 10.1016/j.chabu.2016.04.002

29. Rafid T, O’Leary DD, Dempster KS, Carney M, Wade TJ. Adverse childhood experiences (ACEs) predict increased arterial stiffness from childhood to early adulthood: pilot analysis of the Niagara longitudinal heart study. J Child Adol Trauma. 2020;13:505–514. doi: 10.1007/s40653-020-00311-3

30. Su S, Wang X, Kapuku GK, Treiber FA, Pollock DM, Harshfield GA, McCall WV, Pollock JS. Adverse childhood experiences are associated with detrimental hemodyanmics and elevated circulating Endothelin-1 in adolescents and young adults. Hypertension. 2014;64:201–207. doi: 10.1161/HYPERTENSIONAHA.113.02755

31. Loucks EB, Taylor SE, Polak JF, Wilhelm A, Kalra P, Matthews KA. Childhood family psychosocial environment and carotid intima media thickness: the CARDIA study. Soc Sci Med. 2014;104:15–22. doi: 10.1016/j.socscimed.2013.12.015

32. Hakulinen C, Pulkki-Rä back L, Elovinia M, Kubzansky LD, Jokela M, Hanssen A, et al. Childhood psychosocial cumulative risks and cardiovascular risk factors: a cross-sectional analysis of the Canadian longitudinal study of aging. Can Med Assoc J. 2007;176:1193–1198. doi: 10.1503/cmaj.1070841

33. Kivimäki M, Hintsanen M, Juonala M, Kivimaki M, Josefsson K, Hutri-Kähönen N, et al. Childhood psychosocial cumulative risks and arterial stiffness from childhood to adulthood: the cardiovascular risk in young Finns study. JAMA Pediatr. 2014;168:660–667. doi: 10.1001/jamapediatrics.2014.4121

34. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Mattace-Raso FUS, Schmid M, Boonmann C. Adverse childhood experiences predict increased arterial stiffness from childhood to young adulthood using carotid- femoral pulse wave velocity. J Hypertens. 2017;35:256–265. doi: 10.1093/eurheartj/ehx265

35. Taillieu T, Cheung K, Sareen J. 2016;42:86–96. doi: 10.1093/ije/dys004

36. Afifi TO, MacMillan HL, Boyle M, Taillieu T, Cheung K, Sareen J. Adverse childhood experiences across Europe and North America: a population-based study. J Am Coll Cardiol. 2019;74:1318–1327. doi: 10.1016/j.jacc.2019.09.061

37. Murphy MO, Cohn DM, Loria AS. Developmental origins of cardiovascular disease: impact of early life stress in humans and rodents. Neurosci Biobehav Rev. 2017;74:453–465. doi: 10.1016/j.neubiorev.2016.07.018
37. Horn SR, Leve LD, Levitt P, Fisher PA. Childhood adversity, mental health, and oxidative stress: a pilot study. PLoS One. 2019;14:e0215085. doi: 10.1371/journal.pone.0215085

38. Danese A, McEwen BS. Adverse childhood experiences, allostatic load, allostatic load, and age-related disease. Physiol Behav. 2012;106:29–39. doi: 10.1016/j.physbeh.2011.08.019

39. Ozüik TJ, Toutz RM. Oxidative stress, inflammation, and vascular aging in hypertension. Hypertension. 2017;70:660–667. doi: 10.1161/HYPERTENSIONAHA.117.07802

40. Yeh J-K, Wang C-Y. Telomeres and telomerase in cardiovascular diseases. Genes. 2016;7:58. doi: 10.3390/genes7090058

41. McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. J Hum Hypertens. 2005;19:507–509. doi: 10.1080/jh.19.1011814

42. Nilsson P. Early vascular aging (EVA): consequences and prevention. VHRM. 2008;4:547–552. doi: 10.2147/VHRM.S1094

43. Longhese MP. The role of shelterin in maintaining telomere integrity. Front Biosci. 2012;17:1715–1728. doi: 10.2741/4014

44. Kerszenbaum AL. Telomeres: more than chromosomal non-sticking ends. Mol Reprod Dev. 2000;57:2–3. doi: 10.1002/1098-2795(200006)57:2<2::AID-MRD2>3.0.CO;2-R

45. Blackburn EH, Greider CW, Henderson E, Lee MS, Shampay J, Shippen-Lentz D. Recognition and elongation of telomeres by telomerase. Genome. 1989;31:553–560. doi: 10.1139/g89-104

46. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. 2005;19:2100–2110. doi: 10.1101/gad.134605

47. Samani NJ, van der Harst P. Biological ageing and cardiovascular diseases. Heart. 2008;94:537–539. doi: 10.1136/hrt.2007.136010

48. McDonnell BJ, Yasmin BL, Cockroft JR, Wilkinson IB, Ersalinsky JD, McEniery CM. The age-dependent association between aortic pulse wave velocity and telomere length: age, aortic stiffness and telomere length. J Physiol. 2017;595:1627–1635. doi: 10.1113/JP273689

49. Huzen J. The emerging role of telomere biology in cardiovascular disease. Front Biosci. 2010;15:35–45. doi: 10.2741/4604

50. Jose SS, Bendickova K, Kepak T, Krenova Z, Fric J. Chronic inflammation in immune aging: role of pattern recognition receptor crosstalk between childhood maltreatment and telomere length. Front Immunol. 2017;8:1078. doi: 10.3389/fimmu.2017.01078

51. Reichert S, Ster A. Does oxidative stress shorten telomeres in vivo? A review. Biol Lett. 2017;13:20170463. doi: 10.1098/rsbl.2017.0463

52. Barnes RP, Fouquereil E, Opresko PL. The impact of oxidative DNA damage and stress on telomere homeostasis. Mech Ageing Dev. 2019;177:37–45. doi: 10.1016/j.mad.2018.03.013

53. Glass D, Parts L, Knowles D, Aviv A, Spector TD. No correlation between childhood maltreatment and telomere length. Biol Psychiatry. 2010;68:e21–e22. doi: 10.1016/j.biopsych.2010.02.026

54. Jodcyck S, Ferguson DM, Honword LJ, Pearson JF, Kennedy NA. No association between mean telomere length and life stress observed in a 30 year birth cohort. PLoS One. 2014;9:e97102. doi: 10.1371/journal.pone.0097102

55. Blom HE, Han LKM, Connolly CG, Ho TC, Lin J, LeWinn KZ, Simmons AN, Sacchet MD, Mobayed N, Luna ME, et al. Peripheral telomere length and hippocampal volume in adolescents with major depressive disorder. Transl Psychiatry. 2015;5:e676. doi: 10.1038/tp.2015.172

56. van Ockenburg SL, Bos EH, de Jonge P, van der Harst P, Gans ROB, Rosmalen JGM. Stressful life events and leukocyte telomere attrition in adulthood: a prospective population-based cohort study. Psychol Med. 2015;45:2975–2984. doi: 10.1017/S0033291715000914

57. Verhoeven JE, van Oppen P, Puterman E, Elzinga B, Penninx BWJH. The association of early and recent psychosocial life stress with leukocyte telomere length. Psychosom Med. 2015;77:882–891. doi: 10.1016/j.psymed.2015.02.026

58. Tyrka AR, Price LH, Kao H-T, Porton B, Marsella SA, Carpenter LL. Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging. Biol Psychiatry. 2010;67:531–534. doi: 10.1016/j.biopsych.2009.08.014

59. Tyrka AR, Parade SH, Price LH, Kao H-T, Porton B, Philip NS, Welch ES, Carpenter LL. Alterations of mitochondrial DNA copy number and telomere length with early adversity and psychopathology. Biol Psychiatry. 2016;79:78–86. doi: 10.1016/j.biopsych.2014.12.025

60. Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension. 2001;37:381–385. doi: 10.1161/01.HYP.37.2.381

61. Wang Y-Y, Chen A-F, Wang H-Z, Xie L-Y, Sui K-X, Zhang Q-Y. Association of shorter mean telomere length with large artery stiffness in patients with coronary heart disease. Aging Male. 2011;14:27–32. doi: 10.3109/13685538.2010.529196

62. Strachan T, Goodship J, Chinnery P. Genetics and Genomics in Medicine, Garland Science, Taylor & Francis Group: First; 2015. doi: 10.1161/JAHA.122.026603

63. O’Hara R, Tetedo E, Ludlow A, Huang E, Arosio B, Mari D, Shay E. Quantitative mitochondrial DNA copy number determination using droplet digital PCR with single-cell resolution. Genome Res. 2019;29:1878–1888. doi: 10.1101/gr.250480.119

64. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000;29:222–230. doi: 10.1016/S0891-5849(00)00317-8

65. Yates FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA. 1997;94:514–519. doi: 10.1073/pnas.94.2.514

66. Nilsson P. Early vascular aging (EVA): consequences and prevention. VHRM. 2008;4:547–552. doi: 10.2147/VHRM.S1094

67. Huzen J. The emerging role of telomere biology in cardiovascular disease. Front Biosci. 2010;15:35–45. doi: 10.2741/4604

68. Iliev AN, Czajka A, et al. Mitochondrial DNA content as potential biomarker of mitochondrial dysfunction? Mitochondrion. 2013;13:481–492. doi: 10.1016/j.mito.2012.10.011

69. Foote K, Reinhold J, Yu EPK, Figg NL, Fingan A, Murphy MP, Bennett MR. Restoring mitochondrial DNA copy number preserves mitochondrial function and delays vascular aging in mice. Aging Cell. 2018;17:e12773. doi: 10.1111/ace.12773

70. Rickout KK, Parado SH, Kao H-T, Magnan S, Seifer R, Porton B, Price LH, Tyrka AR. Childhood maltreatment, behavioral adjustment, and molecular markers of cellular aging in preschool-aged children: a cohort study. Psychoneuroendocrinology. 2019;107:261–269. doi: 10.1016/j.psyneuen.2019.05.015

71. Hamczyk MR, Nevado B, Baretton A, Fuster V, Andrés V. Biological aging in hypertension. J Hypertens. 2017;35:910–918. doi: 10.1093/jhypepsy/jxy030

72. Iannarelli et al ACEs, TL, mtDNAcn, and Arterial Stiffness
Iannarelli et al ACEs, TL, mtDNAcn, and Arterial Stiffness

120. Mathur MB, Epel E, Kind S, Desai M, Parks CG, Sandler DP, Khazeni N. Perceived stress and telomere length: a systematic review, meta-analysis, and methodologic considerations for advancing the field. *Brain Behav Immun.* 2016;64:158–169. doi: 10.1016/j.bbi.2016.02.002

121. Jenkins NDM, Rogers EM, Banks NF, Tomko PM, Sciarrillo CM, Emerson SR, Taylor A, Teague TK. Childhood psychosocial stress is linked with impaired vascular endothelial function, lower SIRT1, and oxidative stress in young adulthood. *Am J Physiol Heart Circ Physiol.* 2021;321:H532–H541. doi: 10.1152/ajpheart.00123.2021

122. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Mol Psychiatry.* 2016;21:642–649. doi: 10.1038/mp.2015.67

123. Hostinar CE, Lachman ME, Mroczek DK, Seeman TE, Miller GE. Additive contributions of childhood adversity and recent stressors to inflammation at midlife: findings from the MIDUS study. *Dev Psychol.* 2015;51:1630–1644. doi: 10.1037/dev0000049

124. Knez J, Winckelmans E, Poderoso JJ, Carreras MC. Mitochondrial regulation of cell cycle and proliferation. *Antioxid Redox Signal.* 2012;16:1150–1180. doi: 10.1089/ars.2011.4085

125. Mengel-From J, Thinggaard M, Dalgård C, Kyvik KO, Christensen K, Christiansen L. Mitochondrial DNA copy number in peripheral blood platelet and leukocyte counts. *PLoS One.* 2016;11:e0163770. doi: 10.1371/journal.pone.0163770

126. Vrshek-Schallhorn S, Woltzky-Taylor K, Doane LD, Epstein A, Sumner JA, Mineka S, Zinbarg RE, Craske MG, Isaia A, Hammen C, et al. Validating new summary indices for the childhood trauma interview: associations with first onset of major depressive disorder and anxiety disorders. *Psychol Assess.* 2014;26:730–740. doi: 10.1037/a0038842

127. Goldman EA, Eick GN, Compton D, Kowal P, Snodgrass JJ, Eisenberg DTA, Sterner KN. Evaluating minimally invasive sample collection methods for telomere length measurement. *Am J Hum Biol.* 2018;30:e23062. doi: 10.1002/ajhb.23062

128. Stout SA, Lin J, Hernandez N, Davis EP, Blackburn E, Carroll JE, Glynn LM. Validation of minimally-invasive sample collection methods for measurement of telomere length. *Front Aging Neurosci.* 2017;9:397. doi: 10.3389/fnagi.2017.00397