Investigating the Effects of Markers of Biological Stress on the Association between Adverse Childhood Experiences and Central Artery Stiffness

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Abstract

Adverse childhood experiences (ACEs) have been shown to be associated with an increased risk of cardiovascular disease (CVD). One mechanism by which ACEs may increase CVD risk is through their association with central artery stiffness. Pathways linking ACEs to arterial stiffness have not yet been fully elucidated; however, increased biological stress has been postulated to play a critical role. Recently, two markers have emerged as being potentially useful measures of biological stress—telomere length (TL) and mitochondrial DNA copy number (mtDNAcn). Here, the potential effects of TL and mtDNAcn on the association between ACEs and central artery stiffness were examined. It was hypothesized that TL and/or mtDNAcn would be associated with both ACEs and central artery stiffness, and that these markers would influence the association between ACEs and arterial stiffness. 185 individuals (n = 102 females) aged 19-25 years (mean age 22.5 ± 1.5 years) were included in the current analyses. ACEs were assessed using the CTES 2.0. Central artery stiffness was assessed non-invasively as carotid-femoral pulse wave velocity (cfPWV). TL and mtDNAcn were assessed using qPCR techniques. Multiple linear regression analyses were used to examine the associations between ACEs, TL, mtDNAcn, and cfPWV after adjustment for several covariates. ACEs were independently associated with cfPWV (β = 0.147, p = 0.035). Both TL and mtDNAcn were independently associated with cfPWV (β = -0.169, p = 0.012 and β = -0.525, p = 0.017, respectively). There was no significant association between ACEs and either TL or mtDNAcn (both $p > 0.05$); and neither marker influenced the association between ACEs and cfPWV. Increasing ACEs were associated with a faster cfPWV. This association was not influenced by either TL or mtDNAcn, suggesting that these markers do not provide a link between ACEs and arterial stiffness. Reduced TL and mtDNAcn were also associated with a faster cfPWV. Future studies are required to better understand the association between ACEs, markers of biological stress, and arterial stiffness.

KEY WORDS: central artery stiffness, adverse childhood experiences, telomere length, mitochondrial DNA copy number.

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List of Abbreviations

ACE	Adverse childhood experience
AGE	Advance glycation end-product
AHA	American Heart Association
AIx	Augmentation index
baPWV	Brachial-ankle pulse wave velocity
BP	Blood pressure
CCA	Common carotid artery
cfPWV	Carotid-femoral pulse wave velocity
CHD	Coronary heart disease
CRP	C-reactive protein
ctPWV	Carotid-toe pulse wave velocity
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECM	Extracellular matrix
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
ETC	Electron transport chain
GQ	G-quadruplex
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
IMT	Intima media thickness
ISH	Isolated systolic hypertension
LOX	Lysyl oxidase
LV	Left ventricle
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
mtDNAcn	Mitochondrial DNA copy number
NO	Nitric oxide
ox-LDL	Oxidized low-density lipoprotein
PAD	Peripheral artery disease
PCR	Polymerase chain reaction
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1α
PGC1β	Peroxisome proliferator-activated receptor gamma coactivator 1β
POT1	Protection of telomeres protein 1
PP	Pulse pressure
PPG	Photoplethysmography.
PTT	Pulse transit time
PWV	Pulse wave velocity
qPCR	Quantitative polymerase chain reaction
RAGE	Receptor of AGE
RAP1	*Ras*-related protein 1
RNA	Ribonucleic acid

ROS	Reactive oxygen species
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
SNS	Sympathetic nervous system
SOD-2	Superoxide dismutase-2
TERC	Telomerase RNA complex
TERT	Telomerase reverse transcriptase
TFAM	Transcription factor A, mitochondrial
TIMP	Tissue inhibitor of metalloproteinase
TIN2	TRF1-interacting nuclear factor 2
TL	Telomere length
TPP1	Tripeptidyl peptidase 1
TRF	Terminal restriction fragment
TRF1	Telomeric repeat factor 1
TRF2	Telomeric repeat factor 2
VSMC	Vascular smooth muscle cell

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Chapter I: Introduction

Cardiovascular disease (CVD) is the second leading cause of mortality in Canada (Statistics Canada, 2017), and the leading cause of mortality globally (World Health Organization, 2017). In 2016, an estimated 17.9-million people died from CVD, representing 31% of deaths worldwide (World Health Organization, 2017). Moreover, the economic burden of CVD and CVD-associated mortality on the Canadian healthcare system is substantial, with the costs associated with CVD reaching approximately $13.6-billion (CAD) in Canada in 2010 (Public Health Agency of Canada, 2018). It has been well-established through large population-based longitudinal studies that CV risk begins to develop and accumulate in childhood and adolescence and culminates in disease in adulthood and old age (Dwyer et al., 2013). Indeed, studies such as the Bogalusa Heart Study (BHS), the Cardiovascular Risk in Young Finns Study, and The National Heart, Lung, and Blood Institute Growth and Health Study (NGHS) have found evidence that CV risk factors in childhood are associated with a greater risk of CVD in adulthood (Berenson et al., 1989; Juhola et al., 2011; Li et al., 2004; Thompson et al., 2007). Moreover, large population-based studies, including the Cardiovascular Risk in Young Finns Study and the Health and Social Support Study, have shown that adverse psychosocial experiences during childhood are associated with CV risk and disease in adulthood (Danese et al., 2009; Juonala et al., 2016; Korkeila et al., 2010). Thus, it may be beneficial to examine the etiological beginnings of CV risk in youth and young adulthood as a means to mitigate the risk of CVD later in life and reduce the overall burden of CVD.

The term CVD encompasses all diseases of the heart and blood vessels, including coronary heart disease (CHD), myocardial infarction (MI), stroke, congenital heart disease, and peripheral artery disease (PAD), among others. Several modifiable and non-modifiable risk factors have been linked to CVD, including age, sex, high blood pressure (BP), dyslipidemia, obesity, diabetes mellitus (DM), smoking, and physical inactivity (Nichols & O'Rourke, 2005; Sun & Du, 2017). One way in which these risk factors may contribute to the development of CVD is through their impact on the structure and function of the

central arteries (Nichols & O'Rourke, 2005). Indeed, central artery stiffness has emerged as one of the most important determinants of increased systolic BP, pulse pressure, and pulse wave velocity (SBP, PP, and PWV, respectively) and thus, may be at the core of a host of CV complications and events (Laurent et al., 2006; Nichols & O'Rourke, 2005). Furthermore, central artery stiffness has been shown to be an independent predictor of both CV morbidity and all-cause mortality in several populations (Laurent et al., 2006; Van Bortel et al., 2012).

Central artery stiffness, which describes the stiffness of the aorta and its major branches, is characterized by structural changes within the arterial wall that lead to a marked reduction in arterial compliance and a diminished capacity to buffer cyclic changes in BP (Nichols & O'Rourke, 2005). In healthy individuals, the elastic nature of the central arteries normally exerts a substantial buffering capacity, which functions to maintain a nearly steady flow of blood to the microvasculature despite the pulsatile nature of left ventricular (LV) ejection (Chirinos et al., 2019; Van Bortel & Spek, 1998). With normal aging and in pathology, the central arteries undergo substantial wall remodeling, leading to an impairment in this buffering function (Chirinos et al., 2019; Van Bortel & Spek, 1998). As a result, there is a widening of the PP, a reduction in coronary perfusion, an increase in LV afterload, and an increased transmission of pulsatile energy to the microvasculature (Chirinos et al., 2019). Accordingly, individuals with stiffer arteries are at an increased risk of isolated systolic hypertension (ISH), LV remodeling and dysfunction, and damage to the microvasculature, particularly of the brain and kidneys (Chirinos et al., 2019; Nichols & O'Rourke, 2005).

Although central artery stiffness cannot be measured directly *in vivo*, indices of arterial stiffness can be obtained via the assessment of PWV, a functional parameter influenced by arterial wall stiffness (Laurent et al., 2006). PWV is the rate at which a pulse generated during LV systole propagates throughout a given arterial segment. It is calculated as the ratio of the distance (Δd) between two arterial sites and the time delay (Δt) of the pulse between these sites [i.e., the pulse transit time (PTT)].

As such, PWV is typically expressed in units of metres per second (m/s). The assessment of PWV relies on the principle that stiffer arteries will propagate pressure waves at a faster rate compared to more elastic arteries, thereby providing a straightforward and tangible method for the quantification of central artery stiffness.

Several classical CV risk factors have been associated with a greater degree of central artery stiffness, including aging, sex, high BP, smoking, obesity, and physical inactivity (Ashor et al., 2014; Charakida et al., 2019; Dangardt et al., 2019; Lyle & Raaz, 2017; Mitchell, 2014). Recently, however, adverse childhood experiences (ACEs) have also been postulated to contribute to the progression of arterial stiffness and CVD (Klassen et al., 2016; Korkeila et al., 2010; Su et al., 2014). ACEs have been defined as a collection of traumatic and/or distressing events that occur during childhood (Felitti et al., 1998). Such events can include experiences of childhood maltreatment (i.e., abuse and neglect) and household dysfunction, as well as more targeted experiences of racism, bullying, economic hardship, and community violence, among other stressors (Felitti et al., 1998; Ridout et al., 2018; Suglia et al., 2018). Exposure to childhood adversity is fairly prevalent, with a recent document by Merrick et al. (2018) revealing that nearly 35% of US adults across 23 states had experienced emotional abuse, 28% had experienced physical abuse, and 12% had experienced sexual abuse prior to the age of 18 years (Merrick et al., 2018). Moreover, it was reported that nearly 62% of the study population had experienced at least one ACE and 25% had experienced three or more ACEs (Merrick et al., 2018). ACEs exposures have been consistently associated with higher risks of health-harming behaviours (e.g., smoking, alcohol and drug abuse, overeating, and physical inactivity), mental health disorders, and common chronic diseases, such as CVD and cancer (Bellis et al., 2019; Hughes et al., 2017). Additionally, there is evidence that ACE exposures may ultimately lead to premature mortality (Brown et al., 2009).

One mechanism by which ACEs may contribute to CV risk is through their potential role in the progression of central artery stiffness. To date, few studies have examined the association between

ACEs and increased arterial stiffness (Klassen et al., 2016; Rafiq et al., 2020; Su et al., 2014), and none have examined the impact of ACEs on central artery stiffness, specifically. In one study, Klassen et al. (2016) found that adolescent males aged 10-14 years who had experienced four or more ACEs had greater systemic arterial stiffness compared to those who had experienced less than four ACEs (Klassen et al., 2016). Similarly, Su et al. (2014) found that healthy adolescents and young adults aged 13-29 years who had experienced two or more ACEs had greater peripheral arterial stiffness compared to those who had experienced less than two ACEs (Su et al., 2014). Adversity in childhood has also been associated with increased intima media thickness (IMT) of the common carotid artery (CCA) (Hakulinen et al., 2016; Loucks et al., 2014), which can be related to the elastic properties of the CCA as a measure of local arterial stiffness (Duprez et al., 2000; Laurent et al., 2006). Indeed, Loucks et al. (2014) found evidence that in individuals aged 37-52 years, increasingly adverse childhood family psychosocial environments were positively associated with mean CCA IMT (Loucks et al., 2014). Similarly, Hakulinen et al. (2016) demonstrated that in individuals aged 24-39 years, adverse psychosocial experiences during childhood were associated with a greater CCA IMT later in life (Hakulinen et al., 2016). Taken together, these studies provide evidence that ACEs may contribute to a stiffer arterial phenotype, which may be mediated by different behavioural (e.g., smoking and overeating), mental health (e.g., mood and anxiety disorders), or biological factors (e.g., maladaptive stress response) (Su et al., 2015; Suglia et al., 2018).

In terms of biological factors, mounting evidence suggests that childhood adversity contributes to a maladaptive stress response, which, in turn, may underlie the development of negative CV health trajectories throughout the lifespan (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018). Indeed, maladaptive metabolic and immune responses have been implicated as potential mediators underpinning the ACEs-CV health relationship (Horn et al., 2019; Murphy et al., 2017). It has been hypothesized that childhood adversity disrupts allostasis (i.e., the adaptive processes that maintain homeostasis in response to stressful stimuli) resulting in physiological dysfunction of the stress response

systems (e.g., the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system (SNS), and immune system) (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018). Dysfunction of these systems can result in biological stress (i.e., oxidative stress and chronic low-grade inflammation) via downstream hyperglycemic and inflammatory pathways, which may ultimately lead to deleterious CV health outcomes, such as central artery stiffness and CVD (Guzik & Touyz, 2017; McEniery & Wilkinson, 2005; Ridout et al., 2018; Zhou et al., 2012). Therefore, it may be of interest to examine the potential effects of markers of biological stress on the association between ACEs and increased arterial stiffness, specifically central artery stiffness.

Two markers have recently emerged as being potentially useful surrogate measures of biological stress: telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) (Ridout et al., 2018; Tyrka et al., 2016). Telomeres are specialized nucleoprotein structures located at either end of linear chromosomes (Blackburn et al., 1989; Blackburn, 1991). These structures function to protect, or "cap", the ends of chromosomes from nucleolytic degradation, chromosomal end fusion, and recognition by DNA damage repair response pathways (Kierszenbaum, 2000; Longhese, 2012; Sanders & Newman, 2013). The human telomeric DNA sequence is several kilobases in length and is composed of a tandemly repeated hexameric sequence [i.e., 5'-$(TTAGGG)_n$-3'], which terminates in a single-stranded 3'-overhang (Jansson et al., 2019; Maestroni et al., 2017). With increasing age and number of mitotic divisions, TL progressively shortens (Blackburn et al., 1989; Blackburn, 1991). Once telomeres become critically short, cells undergo replicative senescence and apoptosis, resulting in morphological and functional changes, and loss of tissue function (Blackburn, 1991; de Lange, 2005; Longhese, 2012). TL and the telomere attrition rate are heavily influenced by several endogenous and environmental factors; however, they are particularly susceptible to oxidative stress (Barnes et al., 2019; Jose et al., 2017; von Zglinicki, 2002). Indeed, TL can be reduced by 30-200 base pairs (bp) per cell division, with up to 95% of this loss being linked to oxidative stress (Reichert & Stier, 2017). Prior research has shown significantly reduced TLs in

individuals with stiffer arteries (Benetos et al., 2001; McDonnell et al., 2017), and in those exposed to chronic stress (Epel et al., 2004) and ACEs (Puterman et al., 2016; Tyrka et al., 2010).

Similar to telomeres, mitochondria may also be particularly susceptible to oxidative stress and thus, indices of mitochondrial function may provide an additional marker of biological stress (Ridout et al., 2018). Mitochondria are dynamic organelles that produce energy in the form of ATP (the primary energy source of cells), but also play vital roles in intracellular signaling, lipid and nucleic acid biosynthesis, calcium homeostasis, and reactive oxygen species (ROS) production (O'Hara et al., 2019; Ridout et al., 2018). In contrast to other organelles, the mitochondria house their own genome essential for mitochondrial bioenergetics (Ridout et al., 2018; Strachan et al., 2015). This genome consists of a circular double-stranded 16.6 kb mitochondrial DNA (mtDNA) that encodes for protein components essential to oxidative phosphorylation and mitochondrial protein synthesis (Strachan et al., 2015; Yue et al., 2018). MtDNA is particularly susceptible to oxidative damage and mutations due to its continual exposure to mitochondrial ROS, lack of histone proteins, and limited DNA damage repair capacity (Cadenas & Davies, 2000; Yakes & Van Houten, 1997). As such, the extent of mtDNA damage may be reflected in copy number (mtDNAcn) (Yue et al., 2018). Unlike the nuclear genome that exists in only two copies per diploid cell, various cells can contain hundreds to thousands of copies of mtDNA, depending on their energy demands and oxidative burden (O'Hara et al., 2019; Strachan et al., 2015). Damage to mtDNA has been associated with alterations in mtDNAcn, which may reflect mitochondrial function (or dysfunction) (Malik & Czajka, 2013; Yue et al., 2018), and an increased risk of CVD (Siasos et al., 2018; Yue et al., 2018).

Mitochondrial dysfunction and mtDNAcn have been associated with several CVDs (Yue et al., 2018), including CHD (Liu et al., 2017), heart failure (Huang et al., 2016), and stroke (Ashar et al., 2017), as well as central artery stiffness in mice (Foote et al., 2018). Indeed, human studies have revealed that mean mtDNAcn is reduced in those with various CVDs in middle-age and older adulthood (≥ 45 years),

suggesting that mitochondrial dysfunction may play a key role in the development and progression of CVD (Yue et al., 2018). Interestingly, in mice, Foote et al. (2018) showed that a reduction in mtDNAcn was associated with a reduction in mitochondrial respiration and mitochondrial function, as well as a greater degree of central artery stiffness (Foote et al., 2018), which, as previously described, is considered to be a core component underlying several CVDs (Nichols & O'Rourke, 2005). The authors also found that restoring mtDNAcn through the overexpression of mitochondrial transcription factor A (TFAM) improved both mitochondrial function and arterial elasticity (Foote et al., 2018). Taken together, these human and murine studies provide some evidence that mtDNAcn (as a marker of mitochondrial function) may be reduced in individuals with a greater degree of central artery stiffness; however, this has not yet been examined in humans.

Childhood adversity and psychosocial stress have also been linked to mtDNAcn (Ridout et al., 2019; Tyrka et al., 2016), and have been postulated to play a functional role in mitochondrial dysfunction and the development of disease (Hoffmann & Spengler, 2018; Ridout et al., 2018). Indeed, Tyrka et al. (2016) were the first to demonstrate that experiences of childhood maltreatment were associated with a higher mtDNAcn in individuals aged 18-61 years (Tyrka et al., 2016). This study, however, did not examine whether or not there was a graded response between ACEs and mtDNAcn. Ridout et al. (2019) also revealed a significant association between ACEs and mtDNAcn, such that a greater number of ACEs during the preschool age (i.e., 3-5 years of age) was associated with a greater mtNDAcn after a 6-month follow-up period (Ridout et al., 2019). Importantly, neither of the aforementioned studies examined the long-term effects of an increasingly adverse childhood environment (i.e., the impact of an accumulation of ACEs) on mtDNAcn, which may be more consistent with the development of chronic diseases, such as CVD (Malik & Czajka, 2013; Siasos et al., 2018; Yue et al., 2018).

It has been postulated that mitochondria respond differently to acute versus chronic/persistent stress (Malik & Czajka, 2013). Acutely, the physiological stress response may result in an increased mtDNAcn due to a compensatory increase in mitochondrial biogenesis (Malik & Czajka, 2013; Ridout et al., 2019). This would serve as an adaptive response to provide an individual with the energy needed to respond to a given stressor (Malik & Czajka, 2013; Ridout et al., 2018). Long-term, however, it has been suggested that sustained or repeated activation of the stress response results in a state of persistent biological stress that may eventually lead to a decline in mtDNAcn, reflective of mitochondrial dysfunction (Malik & Czajka, 2013). Accordingly, it may be of interest to assess mtDNAcn (in addition to TL) as a potential marker of biological stress in the context of ACEs and central artery stiffness.

1.1 Study Rationale

Few studies have examined the association between ACE exposures and arterial stiffness, and none have examined the association between ACEs and central artery stiffness. Moreover, the mechanisms by which ACEs may contribute to arterial stiffness have not yet been fully elucidated. Markers of biological stress (i.e., TL and mtDNAcn) have been independently associated with both ACEs and central artery stiffness and thus, may provide a biological link between ACEs and CV health outcomes. Importantly, several questions remain to be answered. First, although several studies have examined the association between TL and ACEs (Bürgin et al., 2019; Ridout et al., 2018), only two studies have explicitly examined the association between mtDNAcn and ACEs (Ridout et al., 2019; Tyrka et al., 2016). Second, only one study has examined the association between TL and central artery stiffness in a healthy young population (McDonnell et al., 2017), and none have examined the association between mtDNAcn and central artery stiffness. Finally, it has been postulated that ACEs may contribute to the progression of central artery stiffness through inducing biological stress (i.e., oxidative stress and inflammation) (Zhou et al., 2012); however, to date, there have been no studies that have examined the potential modulatory effects of markers of biological stress (i.e., TL and mtDNAcn) on the

association between ACEs and arterial stiffness in humans. Therefore, this study will serve to fill gaps in the literature pertaining to telomere and mitochondrial biology with respect to CV health and childhood adversity, as well as expand upon the body of literature that focuses on the ACEs-CV health relationship.

1.2 Hypotheses

The aim of the current study will be to test the following hypotheses:

1) Individuals who have experienced a greater number of ACEs will have a faster carotid-femoral pulse wave velocity (cfPWV) and thus, a greater degree of central artery stiffness.
2) Individuals who have experienced a greater number of ACEs will have a higher burden of biological stress presenting as either:
    a. Shorter mean TL and/or
    b. Lower mean mtDNAcn.
3) Higher burdens of biological stress (i.e., shorter mean TL and/or lower mean mtDNAcn) will be associated with a faster cfPWV.
4) The association between ACEs and central artery stiffness will be mediated by either:
    a. Mean TL and/or
    b. Mean mtDNAcn.

Exploratory analyses will also be performed in order to examine the potential moderating effects of sex, smoking status, BMI, and PA on the postulated associations between ACEs and cfPWV, ACEs and markers of biological stress, and markers of biological stress and cfPWV. Furthermore, the potential moderating effects of TL and mtDNAcn on the postulated association between ACEs and cfPWV will be explored.

Chapter II: Literature Review

2.1 Central Artery Stiffness

In traveling from the heart to the periphery, blood passes through a series of arteries of progressively smaller diameter: elastic arteries, muscular arteries, and arterioles. Together, this collection of vessels makes up the arterial system, with its primary function being to buffer pulsatile LV ejection in order to deliver a near steady flow of oxygen-rich blood to the various tissues and organs of the body (Martini et al., 2018). Commonly referred to as the Windkessel function, the buffering function of the central elastic arteries is of particular importance, as the aorta stores nearly 50% of the LV stroke volume during systole and then forwards this volume to the periphery during diastole at a near constant rate (Belz, 1995). With aging and in pathology, the Windkessel function is progressively diminished and the central arteries become stiff (Laurent et al., 2006; Lyle & Raaz, 2018). Stiffening of the central arteries is detrimental to CV health due to the concomitant rise in SBP and reduction in DBP (i.e., widening of the PP), and the increase in PWV (Laurent et al., 2006; Shirwany & Zou, 2010). These hemodynamic changes have been associated with ISH, LV hypertrophy and dysfunction, poor coronary perfusion, and damage to the microvasculature (Palombo & Kozakova, 2016; Zieman et al., 2005), which, in turn, increase the risk of both stroke and MI (Nichols & O'Rourke, 2005). Accordingly, central artery stiffness has been shown to be an independent predictor of both CV morbidity and all-cause mortality in several populations (Laurent et al., 2006), and has been recommended as a marker of CV risk stratification by the American Heart Association (AHA) (Townsend et al., 2015). In order to better understand what influences arterial stiffness and its consequences, it is important to consider the structure and function of the arterial wall.

2.1.1 Structure and Function of the Arterial System

The walls of arteries are composed of three concentric layers: the tunica adventitia, the tunica media, and the tunica intima (Figure 1). The outermost layer of the arterial wall, the tunica adventitia, is

primarily composed of a thick layer of collagen fibres and fibroblasts, but also contains scattered bands of elastin fibres (Lyle & Raaz, 2018; Martini et al., 2018). The fibres of this layer form a connective tissue sheath around the vessel and blend into those of adjacent tissues, thereby stabilizing and anchoring the blood vessel (Martini et al., 2018). This layer also contains the vasa vasorum and sympathetic nerve innervation (Martini et al., 2018). Deep to the adventitia is the medial layer. This middle layer consists of concentric layers of elastin, collagen fibres, and vascular smooth muscle cells (VSMCs) (Lyle & Raaz, 2018; Martini et al., 2018). The VSMCs of the media encircle the lumen of the vessel and are responsible for maintaining vascular tone in response to various stimuli, including endothelial derived nitric oxide (NO) and endothelin-1 (ET-1) (Lyle & Raaz, 2018). Furthermore, the elastin-collagen ratio of this layer determines the elasticity of a given arterial segment and provides the arterial wall with tensile strength (Lyle & Raaz, 2018). The innermost layer of the arterial wall, the tunica intima, forms the luminal surface of the vessel wall. This layer is composed of a single layer of endothelial cells with an underlying layer of connective tissue and variable levels of elastin fibres (Martini et al., 2018). The endothelium of the intima functions to provide an anti-thrombotic surface for blood flow and produces NO and ET-1 in response to various stimuli in order to alter vascular tone (Lyle & Raaz, 2018). Both the adventitial and medial, and medial and intimal layers are separated by elastic lamellae, which are fenestrated tubes of elastin that further contribute to the elasticity of the arterial wall (Lyle & Raaz, 2018).

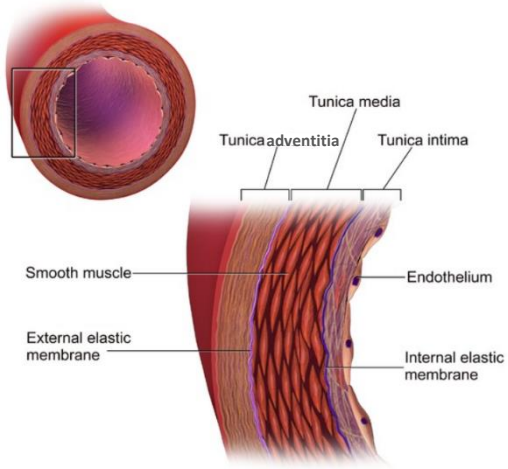

Figure 1. Anatomy of the arterial wall.
Used with permission: Blausen.com staff (2014). "Medical Gallery of Blausen Medical 2014''. *Wikijournal of Medicine* 1(2). DOI:10.15347.wjm.2014.010. ISSN 2002-4436.

The arterial system can be functionally (and structurally) divided into two sub-systems: elastic arteries and muscular arteries (Pugsley & Tabrizchi, 2000; Shirwany & Zou, 2010). The elastic arteries are central vessels located more proximal to the heart, and include the aorta and its major branches (Pugsley & Tabrizchi, 2000). These arteries have thick highly developed medial layers of which elastin is the dominant component (Shirwany & Zou, 2010). Elastin molecules in these arteries are gathered in concentric sheets throughout the thickness of the media and are responsible for the significant buffering capacity of the aorta (Pugsley & Tabrizchi, 2000; Shirwany & Zou, 2010). Despite the important buffering function of central elastic arteries, muscular arteries are the dominant arterial type within the vasculature (Pugsley & Tabrizchi, 2000). Muscular arteries, which include the radial, brachial, and femoral arteries, are medium-sized vessels located more distal to the heart (Pugsley & Tabrizchi, 2000). These arteries have relatively thick medial layers, which are largely composed of VSMCs (Shirwany & Zou, 2010). Indeed, it is the medial layer of muscular arteries that respond to various stimuli in order to constrict or dilate, maintain vascular tone, and closely regulate blood flow (Zieman et al., 2005). Even further distal to the heart, the muscular arteries branch into the arterioles. Arterioles are considerably smaller than the muscular arteries and contain a media composed of only one or two layers of VSMCs

(Martinez-Lemus, 2012; Martini et al., 2018). These vessels are considered the primary resistance vessels, as they provide more than 80% of the resistance to blood flow in the body (Martinez-Lemus, 2012). Accordingly, the arterioles are vital in the regulation of hemodynamics, contributing to both BP control and the delivery of blood to the microvasculature (Martinez-Lemus, 2012).

2.1.2 Hemodynamics of the Central Arteries

In a simplified model of the arterial system, a forward propagated pressure wave is generated in the aorta following LV systole. This pressure wave is then propagated throughout the arterial vasculature to the periphery in order to supply blood to the various tissues and organs of the body. As the pressure wave travels to the periphery, it encounters variations in flow impedance due to the heterogeneity in arterial wall structure throughout the vasculature (Mikael et al., 2017). Such impedance mismatches occur at arterial branch points or further in the periphery where the relatively elastic nature of the aorta gives way to more muscular arteries (Mikael et al., 2017). Once the pressure wave encounters sites of mismatched impedance, two components of the pressure wave arise. The first component is the transmitted pressure wave (Shirwany & Zou, 2010), which is progressively dampened as it propagates along the arterial tree where it eventually reaches the microvasculature (Shirwany & Zou, 2010). The second, quantitatively smaller component, is the reflected (or backward) pressure wave (Shirwany & Zou, 2010). This wave travels retrograde towards the heart where it augments subsequent forward pressure waves (Shirwany & Zou, 2010). When central elastic arteries are compliant, such as in young healthy individuals, the velocity of the forward propagated wave is relatively slow, resulting in a slower reflected wave that arrives back to the aortic root in late systole or early diastole (Mikael et al., 2017). At this point, the reflected wave superimposes on the forward wave, thereby augmenting both the systolic and diastolic pressure waveform (Mikael et al., 2017). BP augmentation serves a key physiologic purpose, as augmentation of SBP results in peak SBP and augmentation of DBP ensures adequate coronary perfusion and thus, preserved myocardial oxygenation (Palombo & Kozakova, 2016).

Moreover, the reflected wave returns a portion of the pulsatile energy to the aorta where it is dissipated, thereby limiting the transmission of pulsatile pressures to the periphery where it can damage the microvasculature (Mikael et al., 2017).

2.1.3 Central Artery Stiffness and Pulse Wave Velocity

The arterial system is under chronic hemodynamic stress over the life course. The oscillatory nature of pulsatile blood flow distends the vessel and induces cyclic circumferential stress, whereas blood flow through the vessel lumen induces shear stress along the endothelium (Lyle & Raaz, 2018). In response to this chronic hemodynamic stress, as well as other stressors (e.g., oxidative and inflammatory stress), the arteries undergo substantial vascular wall remodeling (Lyle & Raaz, 2018; Ungvari et al., 2018). One of the major characteristics observed in this process is the presence of arteriosclerosis, or the thickening and stiffening of the arterial wall (Lee & Oh, 2010; Mikael et al., 2017; Sun, 2015). In the context of central elastic arteries, the most commonly observed structural changes include vessel dilation with wall thickening (i.e., vascular remodeling), as well as a reduction in the elastic property (i.e., stiffening) (Lee & Oh, 2010; Mikael et al., 2017; Sun, 2015).

Central artery stiffness describes the reduced capacity of the aorta and its major branches to expand and contract in response to cyclic changes in BP that occur during the cardiac cycle (Cecelja & Chowienczyk, 2012). Structurally, central artery stiffness is characterized by a marked reduction in the elastin-collagen ratio within the arterial wall extracellular matrix (ECM), and functionally, it is characterized by an increase in several hemodynamic parameters, including SBP, PP, and PWV, as well as a reduction in both compliance and distensibility (Cecelja & Chowienczyk, 2012). Mathematically, the stiffness of a given arterial segment can be defined by Young's elastic modulus:

$$E = \frac{\Delta P}{(\Delta D / D)}$$

where *E* represents Young's elastic modulus, *ΔP* represents the change in intra-arterial pressure (i.e., stress or PP), and *ΔD/D* represents the fractional change in luminal diameter (i.e., strain or distension) (Avolio, 2013). From this equation it can be observed that an increase in PP is associated with a greater elastic modulus and thus, a greater degree of arterial stiffness. In contrast, greater distension is associated with a smaller elastic modulus and thus, a lesser degree of arterial stiffness. Importantly, arteries are generally characterized as exhibiting non-linear stress-strain behaviors (Figure 2) and, as such, the value of *E*, or the stiffness of a given arterial segment, is equal to the slope of the stress-strain curve at any given point (Figure 2) (Avolio, 2013; Nichols & O'Rourke, 2005).

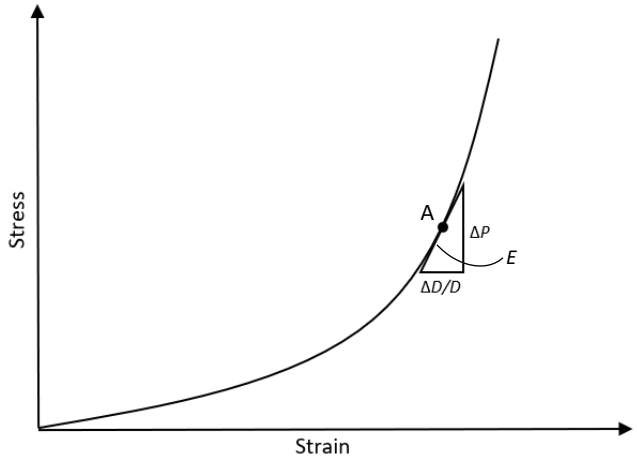

Figure 2. Stress-strain curve of a typical artery. Where the elastic modulus, *E*, is the slope of the curve at point A, Δ*P* is the change in intra-arterial pressure (stress), and Δ*D*/*D* is the change in luminal diameter (strain). Adapted from Nichols & O'Rourke, 2005.

Although arterial stiffness cannot be measured directly *in vivo*, indices of arterial stiffness can be obtained via the assessment of PWV—a functional parameter influenced by arterial wall stiffness (Laurent et al., 2006). PWV is the rate at which a pulse generated during LV systole propagates within an artery. The assessment of PWV relies on the principle that stiffer arteries will propagate pressure waves at a faster rate compared to more elastic arteries, thereby providing a straightforward and tangible

method of quantifying arterial stiffness. This parameter can be described as a function of Young's elastic modulus, arterial diameter, wall thickness, and blood density using the Moens-Korteweg equation:

$$PWV = \left(E \times \frac{h}{D\rho}\right)^{1/2}$$

where *E* is Young's elastic modulus, *h* is arterial wall thickness, *D* is arterial diameter, and *ρ* is blood density (Avolio, 2013). From this equation it can be observed that a larger elastic modulus is associated with a faster PWV and thus, a greater degree of arterial stiffness. This equation also relates arterial wall thickness to arterial stiffness, such that an increased thickness is associated with a faster PWV (i.e., greater arterial stiffness).

2.1.4 Measuring Central Artery Stiffness

As previously described, PWV is the rate at which a pulse (or pressure) wave generated during LV systole propagates within an artery. This parameter is inversely proportional to the elastic property of the arterial wall, in which stiffer arteries will exhibit faster PWVs. PWV is calculated as the ratio of the distance between two arterial sites and the time delay of the pulse between these sites [or the pulse transit time (PTT)]. As such, PWV can be calculated using the following equation:

$$PWV = \frac{\Delta d}{\Delta t}$$

where Δ*d* represents pulse travel distance, expressed in metres (m), and Δ*t* represents the PTT, expressed in seconds (s). Accordingly, PWV is commonly expressed in m/s.

Acquisition of carotid-femoral PWV (cfPWV) is considered the most simple, non-invasive, and reproducible method of determining central artery stiffness (Laurent et al., 2006; Van Bortel et al., 2012). cfPWV is measured along the aortic and aorto-iliac pathway at the sites of the left CCA and left femoral artery (Laurent et al., 2006). The aorta is a major vessel of interest when determining arterial stiffness for two main reasons. First, the thoracic and abdominal aorta make up the largest contribution

to the arterial buffering function and undergo substantial vascular remodeling in response to chronic hemodynamic stress (Lee & Oh, 2010; Mikael et al., 2017). Second, cfPWV has been demonstrated to be an independent predictor of CV morbidity and all-cause mortality in several populations (Ben-Shlomo et al., 2014; Blacher et al., 1999; Laurent et al., 2006). For these reasons, cfPWV is considered the gold-standard measure of central artery stiffness (Laurent et al., 2006; Van Bortel et al., 2012), and as such, the measurement of cfPWV is recommended as a clinical marker of CV risk stratification by the AHA (Townsend et al., 2015). Age-stratified reference values for cfPWV can be observed in Table 1 (The Reference Values for Arterial Stiffness' Collaboration, 2010).

Table 1**.** Distribution of cfPWV (m/s) according to age in the normal population. Adapted from The References Values of Arterial Stiffness' Collaboration, 2010.

Age Stratum (Years):	Mean (± 2 SD):	Median (10-90 pct):
<30	6.2 (4.7-7.6)	6.1 (5.3-7.1)
30-39	6.5 (3.8-9.2)	6.4 (5.2-8.0)
40-49	7.2 (4.6-9.8)	6.9 (5.9-8.6)
50-59	8.3 (4.5-12.1)	8.1 (6.3-10.0)
60-69	10.3 (5.5-15.0)	9.7 (7.9-13.1)
≥70	10.9 (5.5-16.3)	10.9 (8.0-14.6)

*SD, standard deviation; 10 pct, the upper limit of the 10th percentile; 90 pct, the lower limit of the 90th percentile.

Several methods have been developed to assess cfPWV, with the easiest and most widely applied method utilizing techniques of applanation tonometry (Laurent et al., 2006; Pereira et al., 2015). This method employs the use of a high-fidelity strain gauge placed on the tip of a pen-like handheld tonometer, which is applied transcutaneously to an artery (Papaioannou et al., 2003). The pressure in which the tonometer is applied must be light enough as to not occlude the artery, but firm enough to acquire a robust and consistent pulse waveform (Papaioannou et al., 2003). Following pulse wave acquisition, PTT can be measured between the foot of the CCA pulse waveform and foot of the femoral pulse waveform with respect to the R-wave of a gated ECG (i.e., foot-to-foot method) (Figure 3) (Laurent et al., 2006; Yamashina et al., 2002). The distance covered by waves is typically assimilated to the surface distance between the two recording sites and can be measured using an inelastic tape (Figure 3)

(Laurent et al., 2006). Importantly, this measurement is only an estimation of the true distance travelled by a pulse, and it can be affected by how distance was measured as well as other factors, such as abdominal obesity (Laurent et al., 2006; Van Bortel et al., 2012). Furthermore, individual discomfort and difficulty obtaining pressure waveforms in individuals with greater adiposity can also contribute to erroneous cfPWV measurements (Klassen et al., 2016; Laurent et al., 2006).

Measurements of PWV outside of the aortic track, including the brachial (i.e., carotid-radial) PWV and femoral-tibial PWV, have also been demonstrated to be good predictors of CV events; however, these measures have been shown to be inferior to cfPWV, as they are less predictive of CVD and less generalizable (Laurent et al., 2006; Mitchell et al., 2010; Van Bortel et al., 2012). Moreover, these measures do not reflect central artery stiffness (Laurent et al., 2006). Although brachial-ankle PWV (baPWV) and carotid-toe PWV (ctPWV) have been shown to correlate with CV risk and cfPWV, these measures are also not specific to the central elastic arteries (Klassen et al., 2018; Lu et al., 2017).

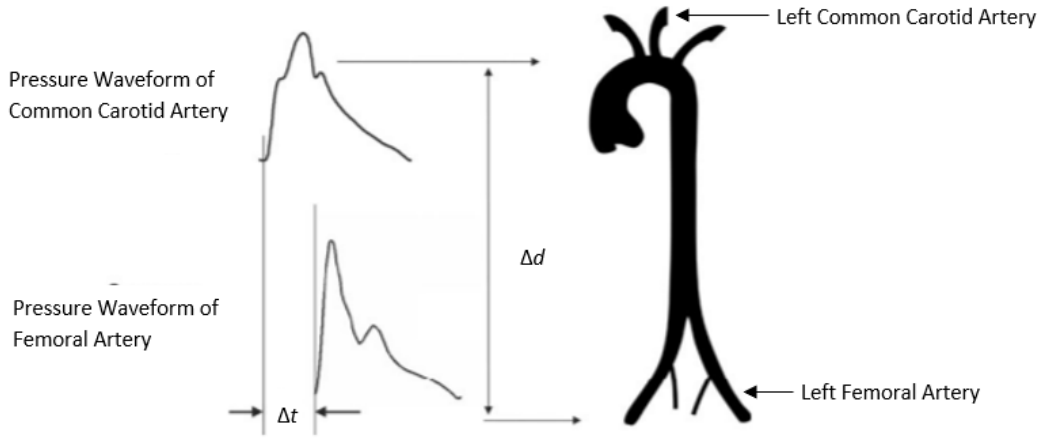

Figure 3**.** Foot-to-foot method for non-invasive measurement of cfPWV. Δ*t* is the PTT between the common carotid and femoral arteries and Δ*d* is the distance between the two arterial sites. Adapted from Laurent et al., 2006 and Laurent & Boutouyrie, 2020.

Several other methods, including photoplethysmography (PPG), doppler ultrasound, magnetic resonance imaging (MRI), and threaded catheterization, have also been utilized to measure PWV and

arterial stiffness; however, these measures are less commonly used in research due to them being less direct (e.g., PPG), laborious, expensive, and technically more complicated (e.g., ultrasound and MRI), and/or invasive (e.g., catheterization) (O'Rourke & Seward, 2006; Pereira et al., 2015).

2.1.5 Hemodynamic Sequalae of Central Artery Stiffness

With increasing central artery stiffness, the velocity of the forward propagated pulse wave (i.e., the PWV) is increased (Palombo & Kozakova, 2016). This increase in PWV results in an earlier reflected wave that returns to the aortic root earlier in the cardiac cycle (i.e., during early systole as opposed to late systole/early diastole) (Palombo & Kozakova, 2016). This, in turn, leads to a significant augmentation in SBP and reduction in DBP (or widening of the PP) (Shirwany & Zou, 2010). This rise in PP is critical in the development of ISH, which is the most common form of hypertension of old age and not uncommon in young- and middle-aged adults (Bavishi et al., 2016). Moreover, the reduction in DBP is associated with a reduction in coronary perfusion and diminished myocardial oxygenation (Palombo & Kozakova, 2016), whereas the rise in SBP may promote the development and rupture of atherosclerotic plaques (Kim & Kim, 2019). Finally, elevated PPs (and ISH) are associated with an increase in LV afterload, hypertrophy, and dysfunction (Palombo & Kozakova, 2016; Zieman et al., 2005), as well as damage to the cerebral and renal microvasculature (Nichols & O'Rourke, 2005). Several mechanisms have been proposed to contribute to the progression of central artery stiffness and the concomitant rise in CV risk (Shirwany & Zou, 2010; Zieman et al., 2005). Importantly, a cfPWV ≥ 10 m/s is considered clinically relevant in the prognosis of negative CV health outcomes (Van Bortel et al., 2012).

2.1.6 Mechanisms of Central Artery Stiffness

2.1.6.1 The Role of Collagen and Elastin

The important buffering function of the central elastic arteries is largely dependent on the composition of the arterial wall ECM, which is composed of several collagens, elastin, gelatin, laminin,

fibronectin, and proteoglycans (Nagase, 2013). These structural components give rise to the integrity and relative elasticity of a given artery, with the latter being directly linked to the elastin-collagen ratio of the arterial wall ECM (Zieman et al., 2005). Normally, the relative content of these proteins are held within a homeostatic range by simultaneous processes of synthesis and degradation; however, dysregulation of this balance can lead to a marked reduction in the elastin-collagen ratio, and may ultimately contribute to the progression of central artery stiffness (Lyle & Raaz, 2018; Zieman et al., 2005).

Elastin has been shown to have an extremely slow turnover rate *in vivo*, and this longevity allows for the accumulation of fragmented and degraded elastin fibres, and disorganized elastin networks (Kohn et al., 2015). As a result, elastin loses its functionality and shifts load bearing onto the much stiffer collagen fibres at lower pressures, which is directly associated with arterial stiffness (Kohn et al., 2015). Moreover, collagen concentrations begin to increase throughout the whole of the arterial wall with aging and in pathology, thereby further shifting the elastin-collagen ratio and contributing to a stiffer vascular phenotype (Kohn et al., 2015). The relative concentrations of elastin and collagen are also influenced by several enzymes, including serine proteases, matrix metalloproteinases (MMPs), and lysyl oxidase (LOX) (Martínez-Revelles et al., 2017; Medley et al., 2003).

2.1.6.2 The Role of Lysyl Oxidase and Matrix Metalloproteinases

Arterial ECM stability is maintained by intra- and intermolecular covalent crosslinking of collagen and elastin fibres, initiated by the copper-dependent amine oxidase, LOX, as well as LOX-like enzymes (Martínez-Revelles et al., 2017). LOX catalyzes the oxidation of lysine residues in elastin, leading to the covalent crosslinking between elastin monomers and the synthesis of desmosine and isodesmosine polymers (Kohn et al., 2015; Martínez-Revelles et al., 2017). These polymers are essential for the formation of cross-linkages between elastin fibres, which, in turn, provides elastin with its elasticity and tensile strength (Kohn et al., 2015). LOX overexpression has been shown to be detrimental to vascular

health as studies have shown that LOX up-regulation increases vascular ROS and contributes to structural alterations in elastin that are associated with arterial stiffness (Martínez-Revelles et al., 2017).

In addition to LOX, MMPs also play a significant role in maintaining arterial ECM stability (Medley et al., 2003). MMPs are a family of zinc-dependent endopeptidases that degrade various components of the arterial wall ECM (Chen et al., 2013). Under normal physiological conditions, MMP activity is regulated at the level of transcription, activation of precursor zymogens, and interactions with other ECM components, such as tissue inhibitors of MMPs (TIMPs), in order to maintain ECM stability (Chen et al., 2013; Raffetto & Khalil, 2008). Imbalance of this system due to, for example, the up-regulation of MMPs, may lead to pathological changes within the arterial wall ECM that are associated with arterial stiffness (Chen et al., 2013; Raffetto & Khalil, 2008). Indeed, several MMPs, including MMPs-1, -2, -3, -7, -8, -9, and -13, have been associated with vascular tissue repair and remodeling (Lyle & Raaz, 2018; Zieman et al., 2005). Of note, MMP-3 may play a particularly important role in central artery stiffness due to its broad substrate spectrum and its ability to activate various other MMPs (e.g., MMPs -1 and -9) (Medley et al., 2003). Accordingly, it has been considered a “master switch” in the initiation of MMP-associated vascular tissue remodeling (Sasamura et al., 2006).

2.1.6.3 The Role of Advanced Glycation End-Products

Advanced glycation end-products (AGEs) are a diverse group of macromolecules that form via the non-enzymatic glycation of proteins and lipids (Senatus & Schmidt, 2017). These molecules have been shown to create cross-linkages between collagen fibres, resulting in stiffer and more turnover-resistant fibres, thereby directly contributing to arterial stiffness (Senatus & Schmidt, 2017; Shirwany & Zou, 2010). Similarly, elastin is sensitive to AGE-associated crosslinking with the consequence of reduced elastic capacity (Shirwany & Zou, 2010). Finally, AGEs have been shown to impact endothelial function by quenching NO and augmenting ROS production by binding to AGE receptors (RAGE) present on the endothelial cell surface (Senatus & Schmidt, 2017). AGE-RAGE binding stimulates oxidative stress via

metabolic and inflammatory mechanisms, which further exacerbate pathological remodeling of the arterial wall (Senatus & Schmidt, 2017).

2.1.6.4 The Role of Endothelial Dysfunction

Endothelial dysfunction, which describes a reduction in vasodilator bioavailability, namely NO, and/or an increase in endothelial-derived vasoconstricting factors, such as ET-1, has also been shown to contribute to arterial stiffness (Hadi et al., 2005; Wilkinson & McEniery, 2004). Indeed, decreased NO and/or increased ET-1 bioavailability may directly contribute to arterial stiffness (and increased BP) by increasing vascular tone and impairing the vasodilatory response (i.e., functional stiffening) (Shirwany & Zou, 2010; Wilkinson & McEniery, 2004). Additionally, endothelial dysfunction has been associated with oxidative stress, as well as a pro-inflammatory and pro-thrombotic state, which may, in part, mediate the association between endothelial dysfunction and arterial stiffness (Hadi et al., 2005; Wilkinson & McEniery, 2004). Importantly, although many studies have established a role of endothelial dysfunction in arterial stiffening, some studies suggest the opposite may also hold true; i.e., arterial stiffness contributes to endothelial dysfunction (Shirwany & Zou, 2010). Accordingly, it has been postulated that a detrimental cycle may exist by which arterial stiffening leads to endothelial disturbances, which, in turn, exacerbate arterial stiffness and so on (Shirwany & Zou, 2010).

2.1.6.5 The Role of Oxidative Stress

Oxidative stress and impaired antioxidant defense mechanisms are believed to be key contributors to CV risk and may also play a significant role in arteriosclerosis (Wu et al., 2014; Zhou et al., 2012). Oxidative stress develops as the result of either an excessive generation of ROS by enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, uncoupled endothelial NO synthase (eNOS), or xanthine oxidase, a reduced antioxidant capacity, or both (Wu et al., 2014). Additionally, ROS are generated as a direct by-product of mitochondrial respiration, particularly at the electron transport chain (ETC), which is a major source of ROS within the vasculature (Raaz et al., 2014;

Wu et al., 2014). Oxidative stress, and notably mitochondrial ROS, have been implicated in the pathogenesis of both atherosclerosis and central artery stiffness (Gioscia-Ryan et al., 2018; Zhou et al., 2012).

Zhou et al. (2012) were the first to demonstrate how mitochondrial oxidative stress over the life course can contribute to aortic stiffening in mice (Zhou et al., 2012). In this study, the researchers used superoxide dismutase 2 (SOD2)-deficient mice to model a state of increased mitochondrial oxidative stress (Zhou et al., 2012). SOD2 normally clears ROS, specifically superoxide, produced during mitochondrial respiration (Kokoszka et al., 2001), and deficiencies in this enzyme have been associated with increased mitochondrial oxidative stress and mitochondrial dysfunction (Kokoszka et al., 2001; Zhou et al., 2012). In their study, Zhou et al. found that increases in mitochondrial oxidative stress (as a result of SOD2 deficiency) were associated with an increase in collagen deposition and fragmented elastin within the aortic wall (Zhou et al., 2012). Additionally, they found that increased oxidative stress was associated with a higher expression and activity of MMP-2 in aortic VSMCs (Zhou et al., 2012). Thus, the authors postulated that oxidative stress likely plays a critical role in the progression of central artery stiffness (Zhou et al., 2012)

Numerous studies in humans have also linked oxidative stress to arterial stiffness (Brinkley et al., 2009; Kawamoto et al., 2016; Patel et al., 2011; Toikka et al., 1999)**.** For example, Toikka et al. (1999) found increased levels of serum oxidized low-density lipoprotein (ox-LDL) to be associated with decreased compliance of both the CCA and ascending aorta in a population of healthy adult males (aged 29-39 years) (Toikka et al., 1999). In terms of PWV, both Brinkley et al. (2009) and Patel et al. (2011) found significant associations between serum ox-LDL and cfPWV in healthy adults (aged > 50 years and 20-70 years, respectively), such that higher levels of serum ox-LDL were associated with a faster PWV, independent of age, sex, BP, and other classical CV risk factors (Brinkley et al., 2009; Patel et al., 2011).

Oxidative stress likely plays a role in the progression of arterial stiffness via changes in both the structure and function of the arterial wall (Brinkley et al., 2009). As previously described, oxidative stress up-regulates the expression and activity of MMPs, which promotes the degradation of the arterial wall ECM and arterial stiffening (Zhou et al., 2012). Oxidative stress-mediated vascular remodeling is also characterized by an inflammatory and fibrotic response, leading to a marked reduction in the elastin-collagen ratio (Zhou et al., 2012) and thickening of the arterial wall (Brinkley et al., 2009). It has also been postulated that oxidative stress-induced arterial wall thickening may be mediated by the atherogenic process, in which oxidative stress-induced endothelial dysfunction, leukocyte adhesion, and VSMC migration and proliferation promote a state of inflammation and atherogenesis that may ultimately lead to intimal-medial thickening and arterial stiffness (Kals et al., 2006; 2008). Additionally, oxidative stress-induced expression of genes that facilitate vascular calcification may also contribute to stiffer arteries (Brinkley et al., 2009). Finally, ROS, specifically superoxide, has been shown to react with NO to form peroxynitrite, which is a strong cytotoxic agent that can cause vascular oxidative damage (Raaz et al., 2014). ROS also quench NO and reduce eNOS expression and activity, thereby reducing NO bioavailability and impairing endothelium-dependent vasodilation (Brinkley et al., 2009).

2.1.7 Factors Associated with Central Artery Stiffness

2.1.7.1 Age, Sex, and Central Artery Stiffness

Aging is considered a major determinant of central artery stiffness (Nichols & O'Rourke, 2005; Sun, 2015). As previously described, structural changes within the arterial wall contribute to central artery stiffness in normal aging (Zieman et al., 2005). These changes are characterized by modest increases in luminal diameter and gradual thickening of the vessel wall (i.e., an increase in IMT), resulting in outward hypertrophic remodeling and an increase in arterial stiffness (Xu et al., 2017). Interestingly, however, the association between aging and central artery stiffness has been observed to be non-linear (McEniery et al., 2005; Oishi et al., 2011). Indeed, both McEniery et al. (2005) and Oishi et

al. (2011) have demonstrated that central artery stiffness increases non-linearly with age, with the most marked increase in stiffness occurring after the age of 50 years (Figure 4) (McEniery et al., 2005; Oishi et al., 2011).

In agreement with these observations, The Reference Values for Arterial Stiffness' Collaboration found that in individuals younger than 50 years of age devoid of any overt CVD, the association between central artery stiffness and age is significant, but weak, with the association becoming stronger with increasing age (The Reference Values for Arterial Stiffness' Collaboration, 2010).

In addition to aging, arterial stiffness may vary by sex (DuPont et al., 2019; Ogola et al., 2018). Indeed, several studies have shown that central artery stiffness differs considerably between males and females in terms of etiology, pathogenesis, and clinical outcomes (DuPont et al., 2019). For example, the Baltimore Longitudinal Study of Aging found that in a population of healthy adults aged 21-94 years, the rate of increase in cfPWV accelerated with advancing age to a greater degree in males compared to females, leading to marked differences in central artery stiffness after the age of 50 years (AlGhatrif et al., 2013). Similarly, the Korean Arterial Aging Study found that cfPWV was significantly faster in males compared to females; however, the difference in cfPWV between sexes was less than 0.15 m/s (Kim et al., 2014). The Reference Values for Arterial Stiffness Collaboration also found a significant difference in cfPWV between males and females; however, this difference was less than 0.1 m/s, resulting in the conclusion that any sex differences in cfPWV are likely negligible (The Reference Values for Arterial Stiffness' Collaboration, 2010). In comparison, two other fairly large longitudinal studies (both $n > 150$) did not report a significant association between sex and cfPWV in normotensive individuals (Benetos et al., 2002; Wildman et al., 2005). Although cfPWV may not vary significantly by sex, it may be irresponsible to exclude sex as a potential covariate influencing central artery stiffness due to inherent differences in CVD risk and development between sexes (Nichols & O'Rourke, 2005).

2.1.7.2 Obesity and Central Artery Stiffness

Obesity is considered a major risk factor for CVD and has been associated with both CV morbidity and all-cause mortality (Li et al., 2017; Wildman et al., 2005). One way in which obesity may adversely affect CV health is through its deleterious effects on the vasculature, by which excess adiposity increases arterial stiffness (Acree et al., 2007; Li et al., 2017; Wildman et al., 2003). Indeed, it has been shown in both adults (Acree et al., 2007; Wildman et al., 2003) and children (Caterini et al., 2017; Wildman et al., 2003) that excess adiposity is associated with a greater degree of central artery stiffness, independent of age, sex, and BP. For example, in a large case-control study involving young and older adults, Wildman et al. (2003) found that higher body mass, body mass index (BMI), and waist-to-hip ratio (WHR) were all associated with a faster cfPWV, independent of age, sex, and BP (Wildman et al., 2003). Of note, it was found that among those aged 20-30 years, obese individuals (BMI > 30) had a mean cfPWV value 0.47 m/s higher than that of their non-obese counterparts (Wildman et al., 2003). Similarly, Caterini et al. (2017) demonstrated that overweight and obese individuals aged 10-18 years had significantly faster cfPWVs compared to healthy-weight controls, independent of age and sex (Caterini et al., 2017). Interestingly, this association held true when cfPWV was measured using MRI techniques (Caterini et al., 2017), which eliminates any overestimation of the distance measurement due to excess adipose tissue when using applanation tonometry techniques (Laurent et al., 2006). Longitudinal associations between obesity and central artery stiffness have also been demonstrated (Brunner et al., 2015; Dangardt et al., 2019). In a 5-year follow-up study as part of the Whitehall II Cohort, Brunner et al. (2015) found a significant longitudinal association between cfPWV and both BMI and WHR in a cohort of individuals aged 35-55 years, independent of age, sex, and BP (Brunner et al., 2015). Similarly, in the Avon Longitudinal Study of Parents and Children, persistent high fat mass during adolescence (9-17 years) was independently associated with increased central artery stiffness in later life (Dangardt et al., 2019). This study also revealed that a return back to normal body mass was

associated with a return to normal cfPWV, suggesting that the effects of obesity on arterial stiffness may be partly reversible (Dangardt et al., 2019). Obesity-associated insulin resistance, inflammation and oxidative stress, and increases in the hormone leptin have been implicated as potential mechanisms linking adiposity with increased arterial stiffness (Leopold, 2013; Li et al., 2017; Safar et al., 2006).

2.1.7.3 Blood Pressure, Heart Rate, and Central Artery Stiffness

High BP and hypertension have conventionally been assumed to antedate and contribute to the progression of central artery stiffness (Mitchell, 2014); however, recent evidence suggests that this may not be the case (Kaess et al., 2012). Indeed, a common interpretation of the known association between arterial stiffness and hypertension is that elevated BP, particularly PP, increases pulsatile wall stress, thereby accelerating elastin degradation and collagen deposition within the arterial wall ECM (Mitchell, 2014). As a result, there is a marked reduction in the elastin-collagen ratio and increased arterial stiffness (Mitchell, 2014; Zieman et al., 2005). In support of this hypothesis, a longitudinal case-control study by Benetos et al. (2002) found that the presence of hypertension was associated with a greater increase in cfPWV over a 6-year period, whereas SBP and DBP within groups (normotensive and hypertensive) were not (Benetos et al., 2002). Conversely, AlGhatrif et al. (2013) found that an elevated SBP at baseline, even in the prehypertensive range (SBP 120-139 mmHg), was associated with a greater increase in cfPWV over a 9-year period, independent of anti-hypertensive medications and other traditional CV risk factors (AlGhatrif et al., 2013). Chen et al. (2016) also reported that elevated SBP preceded central artery stiffness (measured as aortic-femoral PWV) in a sample of middle-aged adults (aged 32-51 years) over a mean follow-up of 7 years; however, the analyses were not adjusted for baseline PWV, which poses an issue as baseline PWV may influence PWV at follow-up (Chen et al., 2016). Similarly, Li et al. (2004) reported that childhood and lifetime burden of SBP was associated with adult baPWV; however, these analyses did not adjust for baseline (childhood) baPWV (Li et al., 2004).

In contrast, several longitudinal studies have demonstrated that greater arterial stiffness in normotensive individuals at baseline is associated with accelerated BP progression and an increased risk of incident hypertension (Kaess et al., 2012; Wu et al., 2019). For example, using a sample of individuals from the Framingham Heart Study, Kaess et al. (2012) demonstrated that baseline cfPWV was independently associated with SBP, PP, and incident hypertension at a 7-year follow-up (Kaess et al., 2012). Moreover, the authors revealed that higher initial BPs were not predictive of greater arterial stiffness at follow-up (Kaess et al., 2012). Similarly, a large community-based study found that baPWV at baseline was associated with subsequent BP at a 6-year follow-up, rather than the opposite (Wu et al., 2019). Importantly, both of the aforementioned studies adjusted their analyses for baseline measurements of arterial stiffness (Kaess et al., 2012; Wu et al., 2019).

The relationship between central artery stiffness and BP is likely complex. Based on the current literature, it has been proposed that the association between central artery stiffness and BP may be best described as "feed-forward"; i.e., central artery stiffness likely contributes to a rise in BP, which, in turn, exacerbates central artery stiffness and so on (AlGhatrif et al., 2013; Safar et al., 2018). Regardless, it is evident that BP and arterial stiffness are largely co-dependent, making it important to consider BP in the assessment of PWV (Avolio, 2013; Nichols & O'Rourke, 2005).

In addition to BP, heart rate (HR) has also been associated with central artery stiffness (Benetos et al., 2002; Logan & Kim, 2016; Tan et al., 2016). In studies that have manipulated HR through cardiac pacing, a significant and positive association between HR and cfPWV has been demonstrated (Lantelme et al., 2002; Millasseau et al., 2005; Tan et al., 2016). Importantly, the effects of HR on cfPWV were shown to be independent of both age and BP, albeit with a much smaller influence compared to the latter two variables (Lantelme et al., 2002; Tan et al., 2016). The Reference Values for Arterial Stiffness' Collaboration (2010) also found that HR was a significant predictor of cfPWV in a large population of Europeans (n > 11,000), independent of age, sex, and MAP (The Reference Values for Arterial Stiffness'

Collaboration, 2010). However, again, HR had a much smaller influence on cfPWV compared to age and MAP (The Reference Values for Arterial Stiffness' Collaboration, 2010). In a longitudinal analysis, Benetos et al. (2002) found that in hypertensive individuals (mean age 57 years at baseline), but not normotensive individuals, HR was a significant predictor of cfPWV at both baseline and at a 6-year follow-up (Benetos et al., 2002). Conversely, Logan and Kim (2016) found that resting HR was a significant predictor of cfPWV, independent of age, BMI, and MAP in a population of normotensive adults aged 21-60 years (mean age 39.6 ± 9.9 years) (Logan & Kim, 2016). Millasseau et al. (2005) also found HR to be a significant predictor of cfPWV in a sample of normotensive older adults (mean age 62.0 ± 17.0 years); however, their sample size was relatively small (*n* = 11) (Millasseau et al., 2005). Finally, in a sample of normotensive adults aged 40-93 years, Tan et al. (2016) found that a 10 bpm increase in HR was associated with a 0.16-0.20 m/s increase in cfPWV, independent of age, sex, and BP (Tan et al., 2016). Accordingly, the authors concluded that HR should be considered a significant contributing factor to central artery stiffness (Tan et al., 2016; 2018)

2.1.7.4 Lifestyle Factors and Central Artery Stiffness

Lifestyle factors, including high dietary salt intake (Avolio et al., 1985, 1986), low physical activity (Cavero-Redondo et al., 2019; Zhang et al., 2018), and smoking (Charakida et al., 2019) have also been associated with central artery stiffness. Indeed, two large population-based studies provide support for a BP-independent association between high dietary sodium intake and central artery stiffness (Avolio et al., 1985, 1986). Avolio et al. (1985) first demonstrated that cfPWV of individuals who consumed less dietary salt were consistently slower than those who consumed higher levels of salt, independent of age and BP status (Avolio et al., 1985). Avolio et al. (1986) reproduced this finding in a second study involving a normotensive population in which they found that low-salt diets were associated with slower cfPWVs, independent of age and BP status (Avolio et al., 1986). More recently, a meta-analysis conducted by D'Elia et al. (2018) demonstrated that a moderate reduction in dietary sodium intake

reduced central artery stiffness, and that this association is at least in part independent from concomitant changes in BP (D'Elia et al., 2018).

Physical activity (PA) level has also been associated with central artery stiffness (Ashor et al., 2014; Cavero-Redondo et al., 2019; Zhang et al., 2018). Indeed, a large meta-analysis by Ashor et al. (2014) found that regular aerobic exercise was significantly associated with a reduction in cfPWV (Ashor et al., 2014). Similarly, Zhang et al. (2018) revealed that aerobic exercise alone or combined with resistance training was associated with a reduction in cfPWV (Zhang et al., 2018). Interestingly, it has been reported that, in comparison to aerobic training alone, resistance training alone is not associated with reductions in arterial stiffness (Ashor et al., 2014; Evans et al., 2018), and may actually contribute to increased arterial stiffness (Miyachi, 2013). Finally, modest increases in PA levels have been shown to reduce central artery stiffness (Cavero-Redondo et al., 2019). In a recent meta-analysis by Cavero-Redondo et al. (2019), steps per day and cfPWV were demonstrated to be significantly inversely associated, such that each 1,000 step per day increase was associated with a 0.17 m/s reduction in cfPWV (Cavero-Redondo et al., 2019). Together, these findings provide evidence that PA may be a major modifiable risk factor associated with central artery stiffness.

Finally, cigarette smoking has been associated with central artery stiffness (Charakida et al., 2019). In one review, it was found that acute, chronic, and passive smoking were all associated with a faster cfPWV (Doonan et al., 2010). Similarly, both Kim et al. (2005) and Binder et al. (2008) found that smokers had a greater degree of central artery stiffness compared to non-smokers (Binder et al., 2008; Kim et al., 2005), and Charakida et al. (2019), reported a significant dose-response relationship between number of cigarettes smoked and increasing cfPWV (Charakida et al., 2019). However, not all studies demonstrated a significant association between smoking status and cfPWV (Camplain et al., 2016; Mahmud & Feely, 2003; Rehill et al., 2006). It has also been reported that smoking cessation may reduce

the stiffness of central arteries, suggesting that the effects of smoking may in part be reversible (Charakida et al., 2019; Doonan et al., 2010; Rehill et al., 2006).

2.1.7.5 Adverse Childhood Experiences, Central Artery Stiffness, and Cardiovascular Disease

There is growing evidence that suggests early life CV risk factors may contribute to the development of CVD in adulthood. Such findings include those of obesity, dyslipidemia, and high BP during childhood predicting atherosclerosis (Berenson et al., 1998), thickened CCA IMT (Li et al., 2004), and overall CV risk (Juhola et al., 2011; Thompson et al., 2007) in adulthood. Moreover, exposures to childhood adversity have been shown to predict CV risk in adulthood (Juonala et al., 2016; Korkeila et al., 2010). Indeed, exposures to adverse childhood experiences (ACEs) have been shown to have profound impacts on health trajectories over the life course (Basu et al., 2017). For example, the landmark Adverse Childhood Experiences (ACE) Study published in 1998 was the first to report a significant dose-response relationship between the number of ACEs exposures and poor health outcomes in adulthood (Felitti et al., 1998). Indeed, data from this study revealed a significant dose-response relationship between the number of ACEs exposures and risk of CHD in adulthood (Dong et al., 2004). Subsequent studies have bolstered this finding, revealing that ACEs are associated with an increased risk of several CV events, including hypertension, CHD, MI, stroke, and CVD-associated mortality (Basu et al., 2017; Su et al., 2015; Suglia et al., 2018). One mechanism by which ACEs may contribute to CV risk is through their potential role in the progression of central artery stiffness (Laurent et al., 2019; Nilsson, 2008). To date, few studies (Klassen et al., 2016; Su et al., 2014) have examined the association between ACEs and increased arterial stiffness, and none have examined the impact of ACEs on central artery stiffness. Thus, it may be prudent to examine the association between ACEs and central artery stiffness (i.e., cfPWV) as a potential link between childhood adversity and CV risk later in life.

2.2 Adverse Childhood Experiences

2.2.1 Definition and Burden

An increasing number of studies have identified the long-term effects of ACEs on health trajectories over the life course (Bellis et al., 2018; Hughes et al., 2017). ACEs have been defined as a collection of traumatic and/or distressing events that occur during childhood (Felitti et al., 1998, 2002). Such events can include experiences of childhood maltreatment (e.g., physical, sexual, or emotional abuse or neglect) and household dysfunction (Felitti et al., 1998, 2002), as well as more targeted experiences of racism, bullying, economic hardship, and community violence, among other stressors (Ridout et al., 2018; Suglia et al., 2018). ACEs typically do not occur in isolation, but instead tend to co-occur, such that if an individual has experienced one type of ACE (e.g., household dysfunction), it is likely that they have experienced some additional ACE (e.g., physical or emotional abuse) (Chartier et al., 2010; Felitti et al., 1998; S. McDonald et al., 2015). Indeed, Felitti et al. (1998) revealed that for individuals reporting an exposure to at least one ACE, the probability of exposure to any additional ACE ranges from 65-93% (Felitti et al., 1998). Moreover, in a large US-based study (n > 210,000), it was found that approximately 38% of US adults had experienced at least two ACEs (Merrick et al., 2018). Childhood adversity has been consistently linked to higher risks of health-harming behaviours (e.g., smoking, alcohol and drug abuse, overeating, and physical inactivity), mental health disorders, and chronic diseases, such as CVD and cancer (Bellis et al., 2019; Hughes et al., 2017). Additionally, there is evidence that ACEs exposures are linked to premature mortality (Brown et al., 2009).

It is largely thought that childhood maltreatment and household dysfunction are rare occurrences and that their effect on the overall health of a population is minimal; however, this is not the case (Wade et al., 2019). Indeed, exposure to childhood adversity has been shown to be fairly prevalent, with a recent document by Merrick et al. (2018) revealing that nearly 35% of US adults across 23 states had experienced emotional abuse, 28% had experienced physical abuse, and 12% had

experienced sexual abuse prior to the age of 18 years (Merrick et al., 2018). Moreover, it was reported that nearly 62% of the study population had experienced at least one ACE and 25% had experienced three or more ACEs (Merrick et al., 2018). Similarly, the prevalence of childhood adversity is high amongst Canadians (Afifi, 2016; S. McDonald et al., 2015). Recent retrospective Canadian data have estimated that the prevalence of child abuse ranges anywhere from 27-32% (Afifi, 2014; McDonald et al., 2015), and that the prevalence of household dysfunction is nearly 49% (S. McDonald et al., 2015). In Ontario in 2010, the Ontario Health Survey also reported that 72% of Ontarians had experienced at least one ACE, and 16% had experienced three or more ACEs (Chartier et al., 2010). Moreover, 26% of Ontarians had experienced some form of physical abuse and 9% had experienced some form of sexual abuse prior to the age of 18 years (Chartier et al., 2010). From a public health standpoint, children with higher ACEs exposures tend to utilize health-care much more as adults compared to those with no ACE exposures (Chartier et al., 2010; Su et al., 2014). Indeed, a recent analysis estimated that the total annual health care costs attributable to ACEs were $748-billion (USD) in North America, and more than 75% of these costs were attributed to individuals exposed to two or more ACEs (Bellis et al., 2019).

2.2.2 Adverse Childhood Experiences and Cardiovascular Outcomes

As previously described, the landmark ACE Study was the first to publish reports of a significant dose-response relationship between the number of ACEs exposures and deleterious health outcomes in adulthood (Felitti et al., 1998). Indeed, it was this study that revealed a positive association between ACEs exposures and risk of CHD, with those experiencing at least one ACE having a 1.3-1.7-fold increased risk of CHD compared to those with no ACEs exposures (Dong et al., 2004). These initial findings continue to be validated by a growing body of literature demonstrating the long-term effects of ACEs on CV health and disease (Hakulinen et al., 2016; Juonala et al., 2016; Korkeila et al., 2010), with several documents demonstrating significant associations between ACEs and an increased risk of CVD and CVD-associated mortality (Basu et al., 2017; Su et al., 2015; Suglia et al., 2018). Additionally, ACEs have been

associated with high BP and hypertension (Gooding et al., 2016; Stein et al., 2012; Su et al., 2015), as well as indices of atherosclerosis and arterial stiffness (Hakulinen et al., 2016; Klassen et al., 2016; Loucks et al., 2014; Su et al., 2014; Rafiq et al., 2020).

It has been postulated that ACEs may contribute to CV risk through their potential role in the progression of central artery stiffness; however, few studies have examined the association between ACEs and increased arterial stiffness, and none have examined the impact of ACEs on central artery stiffness as measured by cfPWV. In one study, Klassen et al. (2016) found that adolescent males aged 10-14 years who had experienced four or more ACEs had greater systemic arterial stiffness compared to those who had experienced less than four ACEs (Klassen et al., 2016). Similarly, Su et al. (2014) found that healthy adolescents and young adults aged 13-29 years who had experienced two or more ACEs had greater peripheral arterial stiffness compared to those who had experienced less than two ACEs (Su et al., 2014). Bomhof-Roordink et al. (2015) also found a significant association between childhood trauma and aortic stiffness in individuals aged 20-66 years; however, this study utilized augmentation index (AIx), which is both an indirect and less accurate measure of arterial stiffness compared to cfPWV (Laurent et al., 2006). Finally, in a recently published study, Rafiq et al. (2020) found that individuals who had experienced four or more ACEs had a greater increase in systemic arterial stiffness (i.e., ECG-toe PWV) compared to those who had experienced less than four ACEs over a 9-year period from childhood (mean age 12 ± 1 years) to young adulthood (mean age 21 ± 1 years) (Rafiq et al., 2020). Importantly, this increase in systemic arterial stiffness was similar in both males and females and remained significant after adjustment for sex and changes in HR, SBP, BMI, and PA (Rafiq et al., 2020).

Additionally, Loucks et al. (2014) found evidence that in individuals aged 37-52 years, increasingly adverse childhood family psychosocial environments were positively associated with mean CCA IMT (Loucks et al., 2014). This finding was replicated by Hakulinen et al. (2016), in which an increasing number of ACEs exposures was associated with an increased CCA IMT and a greater

progression of CCA IMT over a 6-year follow-up period in individuals aged 24-39 years (Hakulinen et al., 2016). Importantly, CCA IMT is not only a marker of subclinical atherosclerosis (Bauer et al., 2012), but it can also be examined in relation to the elastic properties of the CCA as a measure of local arterial stiffness using the Moens-Korteweg equation (Cecelja & Chowienczyk, 2012; Duprez et al., 2000; Laurent et al., 2006). Finally, a study by Juonala et al. (2016) revealed a longitudinal association between childhood adversity and increased levels of coronary artery calcification in adulthood, independent of conventional CV risk factors (Juonala et al., 2016). Taken together, these findings demonstrate a susceptibility of the arterial vasculature to undergo remodeling and wall stiffening in response to childhood adversity. No studies to date have examined the association between ACEs and central artery stiffness measured as cfPWV—the gold-standard measure of arterial stiffness and an independent predictor of both CV morbidity and all-cause mortality (Laurent et al., 2006; Van Bortel et al., 2012).

2.2.3 Mechanisms Linking Adverse Childhood Experiences and Cardiovascular Outcomes

Although the exact mechanisms linking childhood adversity to deleterious CV health outcomes have not yet been fully elucidated, at least three pathways are commonly identified—behavioural, mental health, and biological pathways (Su et al., 2015; Suglia et al., 2018). First, childhood adversity is associated with negative health behaviours, such as smoking, alcohol and drug abuse, disordered eating, and physical inactivity, which are known to increase CV risk (Felitti et al., 1998; Su et al., 2015). Indeed, Felitti et al. (1998) first hypothesized that the link between ACEs and disease in adulthood resulted from those being exposed to ACEs engaging in negative health behaviours (e.g., smoking, overeating) as a way to cope with the stress of childhood adversity (Anda, 1999; Felitti et al., 1998). Support for this hypothesis came from Anda (1999), who found that those exposed to five or more ACEs were at a 5-fold increased risk of early smoking initiation compared to those exposed to no ACEs—a result that was attributed to the psychoactive benefits of nicotine in the regulation of stress (Anda, 1999). Conversely, smoking is associated with both an increase in central artery stiffness and overall CV risk (Charakida et

al., 2019; Doonan et al., 2010), and thus, may influence the association between ACEs and CVD in adulthood (Anda, 1999; Suglia et al., 2018). Similarly, physical inactivity and disordered eating may also contribute to the association between ACEs and CVD (Su et al., 2015; Suglia et al., 2018). Childhood adversity has been associated with both inactivity and overeating (Su et al., 2015), which, may increase the risk of obesity and central artery stiffness, and may ultimately influence CV risk in adulthood (Juonala et al., 2011; Suglia et al., 2018).

Poor mental health status has also been proposed as a mechanism linking ACEs to deleterious CV health outcomes in adulthood (Su et al., 2015; Suglia et al., 2018). Indeed, it has been shown that childhood adversity is associated with an increased risk of several mental health conditions, including mood and anxiety disorders (e.g., major depressive disorder and general anxiety disorder), as well as posttraumatic stress disorder (PTSD) (Hughes et al., 2017; Suglia et al., 2018). In a 32-year prospective longitudinal study by Danese et al. (2009), it was revealed that individuals exposed to childhood adversity were at an elevated risk of depression in adulthood (Danese et al., 2009). Similarly, in a representative sample of US children and young adults, Copeland et al. (2007) found that children displaying PTSD symptoms were more likely to have a history of ACEs exposures (Copeland et al., 2007). Both mood disorders and PTSD have been associated with incident CVD (Su et al., 2015; Suglia et al., 2018), with a recent statement by the AHA positing the former as an important risk factor predisposing youth to accelerated atherosclerosis and early CVD (Goldstein et al., 2015). Prospective population-based observational studies have also found PTSD to be associated with an increased risk of CVD (Kubzansky et al., 2007; Vaccarino et al., 2013); however, whether and how much of the association between ACEs and CVD can be explained by PTSD is unclear, largely because most studies have included traumatic events over the life course and many focus on veteran/military service-related trauma, not specifically childhood adversity (Suglia et al., 2018). Lastly, a recent meta-analysis by Basu et al. (2017) found that mental health conditions were partial mediators in the association between childhood

maltreatment and CVD (Basu et al., 2017), thereby supporting the role of mental health conditions in the association between ACEs and CVD. Interestingly, any potential mediating effects of mental health disorders on the association between ACEs and arterial stiffness have not yet been examined.

Finally, mounting evidence suggests that childhood adversity contributes to a maladaptive stress response, which, in turn, may underlie the development of CV health trajectories throughout the lifespan (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018). Indeed, maladaptive metabolic and immune responses have been implicated as potential mediators underpinning the ACEs-CV health relationship (Horn et al., 2019; Murphy et al., 2017). It has been hypothesized that childhood adversity disrupts allostasis (i.e., the adaptive processes that maintain homeostasis in response to stressful stimuli), resulting in physiological dysfunction of the stress response systems (e.g., HPA axis, SNS, and immune system) that may contribute to pathology (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018). In normal physiology, the stress response is an essential adaptive mechanism in response to dynamic and changing external stimuli (Danese & McEwen, 2012). Moreover, the stress response, in the short-term, may be particularly beneficial to children living in high-risk environments (Ridout et al., 2018; Suglia et al., 2018). For example, in response to stressful stimuli, the SNS responds within seconds to release epinephrine and norepinephrine, which rapidly signals multiple physiological changes, including increasing HR and BP, splanchnic vasoconstriction, and bronchodilation, thereby priming the body for “fight or flight” (Ridout et al., 2018). Similarly, the HPA axis plays a critical role in the stress response; however, at a much slower rate (Ridout et al., 2018). Activation of the HPA axis in response to stressful stimuli culminates in the release of cortisol, which is mainly responsible for promoting the production of glucose necessary for the energy metabolism that fuels the response to the stressor (Ridout et al., 2018). However, because ACEs do not often occur in isolation (Austin, 2018; Felitti et al., 1998), and due to their associated allostatic load (Danese & McEwen, 2012), chronic activation of the stress response systems can occur, resulting in physiological dysfunction (Danese & McEwen, 2012).

Dysfunction of the stress response systems can result in biological stress (i.e., chronic low-grade inflammation and oxidative stress) via downstream inflammatory and hyperglycemic pathways, which may ultimately contribute to deleterious CV health outcomes, such as central artery stiffness and CVD (Guzik & Touyz, 2017; McEniery & Wilkinson, 2005; Ridout et al., 2018; Zhou et al., 2012). Therefore, it may be prudent to examine the mediating effect of markers of biological stress on the association between ACEs and central artery stiffness. Recently, two biological markers have emerged as being potentially useful surrogate measures of biological stress—telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) (Ridout et al., 2018; Tyrka et al., 2016).

2.3 Markers of Biological Stress

2.3.1 Telomeres

Telomeres are specialized nucleoprotein structures located at either end of linear chromosomes (Figure 5) (Blackburn et al., 1989; Blackburn, 1991). These structures function to protect, or "cap", the ends of chromosomes from nucleolytic degradation, chromosomal end fusion, and recognition by DNA damage repair response pathways (Kierszenbaum, 2000; Longhese et al., 2012; Sanders & Newman, 2013). Additionally, telomere capping is required for the complete replication of chromosomal ends and thus, genomic stability (Longhese, 2012). The human telomeric DNA sequence is several kilobases (kb) in length and is composed of a tandemly repeated hexameric sequence [i.e., 5'-$(TTAGGG)_n$-3'], which terminates in a single-stranded 3'-overhang (Jansson et al., 2019; Maestroni et al., 2017). With increasing age and number of mitotic divisions, TL progressively shortens (Blackburn et al., 1989; Blackburn, 1991). Once telomeres become critically short (i.e., reach their Hayflick limit), cells undergo replicative senescence and apoptosis, resulting in morphological and functional changes that may culminate in loss of tissue function (Blackburn, 1991; de Lange, 2005; Longhese, 2012). TL and the telomere attrition rate are heavily influenced by several endogenous and environmental factors, and are particularly susceptible to biological stress (i.e., inflammation and oxidative stress (Barnes et al., 2019;

Jose et al., 2017; von Zglinicki, 2002). Indeed, TL can be reduced by 30-200 bp per cell division, with anywhere from 33-95% of this loss being related to biological stress (Reichert & Stier, 2017). Prior research has demonstrated significantly shortened telomeres in individuals with stiffer arteries (Benetos et al., 2001; McDonnell et al., 2017), and in those exposed to chronic stress and ACEs (Epel et al., 2004; Puterman et al., 2016; Tyrka et al., 2010).

2.3.1.1 Structure and Function

As previously described, telomeres are specialized structures that cap the ends of linear chromosomes. In humans, TL is heritable, relatively short, and highly variable (Benetos et al., 2001). Human telomeric DNA consists of a 5-15 kb long double-stranded sequence that, at the distal end, terminates in a single-stranded 3'-overhang that is typically 50-500 bp in length (Figure 5) (De Meyer et al., 2018; Jansson et al., 2019; Maestroni et al., 2017). At the proximal end, telomeres are separated from adjacent DNA by sub-telomeric regions, which are composed of a dynamic patchwork of sequences containing high-density repeats, few genes, and various methylation sites (De Meyer et al., 2018; Hu et al., 2019). Importantly, the tandemly repeated telomeric sequence is non-coding and guanine-rich (G-rich) (Blackburn, 1991; Blackburn et al., 1989). The G-rich nature of the telomeric sequence, along with the 3'-overhang, permit the folding of telomeres into at least two different structures: T-loops and G-quadruplexes (Shay & Wright, 2019; Xin et al., 2008). These structures, in conjunction with a group of telomere-specific proteins, aid in the regulation of TL and are required for the proper capping of chromosomes (de Lange, 2005, 2009; Shay & Wright, 2019; Xin et al., 2008). Moreover, these structures distinguish telomeres from double-stranded DNA breaks and are lost upon critical telomere attrition, thereby inducing replicative senescence and apoptosis (De Meyer et al., 2018).

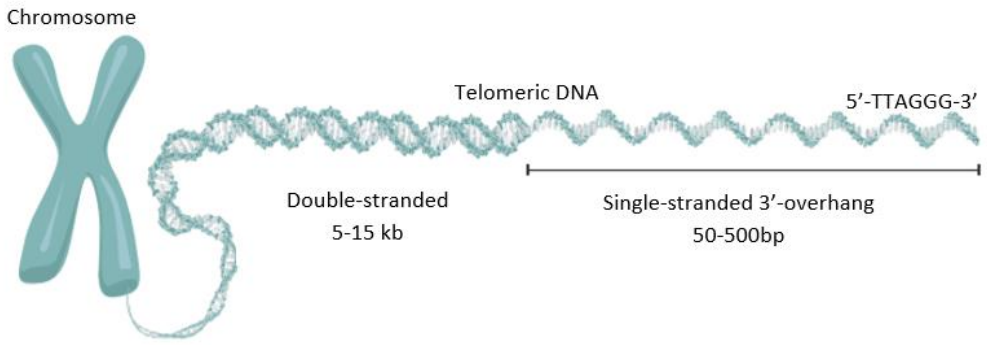

Figure 5. Telomere structure. Adapted from Sandin & Rhodes, 2014.

G-Quadruplexes and T-Loops:

The G-rich nature of telomeric DNA promotes the formation of G-quadruplex (GQ) structures (Rice & Skordalakes, 2016a). GQ structures are formed when four guanine residues come together via hydrogen-bonding to form planar structures that further stack together to yield stable DNA folds (Jansson et al., 2019; Moye et al., 2015) (Figure 6). These structures have been implicated in TL regulation, as they have been demonstrated to impede both telomerase activity (Jansson et al., 2019; Moye et al., 2015) and nucleolytic degradation at chromosomal ends (Rice & Skordalakes, 2016a).

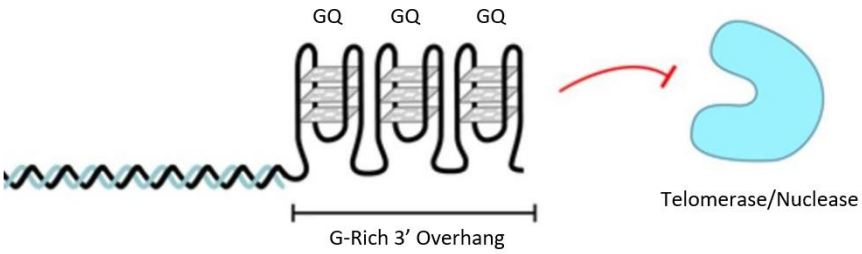

Figure 6. Structure of the telomeric G-quadruplex (GQ). GQ structures prevent nucleolytic degradation and telomerase activity at the telomeric ends, thereby aiding in the regulation of TL. Adapted from Rhodes & Lipps, 2015.

In contrast to GQ structures, T-loops are generated when the G-rich 3’ overhang invades double-stranded telomeric DNA to form a lariat-like loop structure (Rice & Skordalakes, 2016a; Shay & Wright, 2019) (Figure 6). T-loop formation is promoted and stabilized by the shelterin complex, which is

composed of several telomere-specific proteins (Hu et al., 2019; Rice & Skordalakes, 2016a). Within the T-loop structure, the G-rich 3' overhang base pairs with the cytosine-rich strand of double-stranded telomeric DNA, thereby displacing the G-rich strand and forming a secondary loop at the base of the T-loop, which is referred to as the D-loop (de Lange, 2009) (Figure 7). Similar to GQ structures, T-loops provide a regulatory mechanism for the maintenance of TL. Additionally, T-loops aid in the telomeric capping function, as they hide the ends of chromosomes from exposure to nucleolytic degradation and recognition by DNA damage response pathways (de Lange, 2009; Rice & Skordalakes, 2016b).

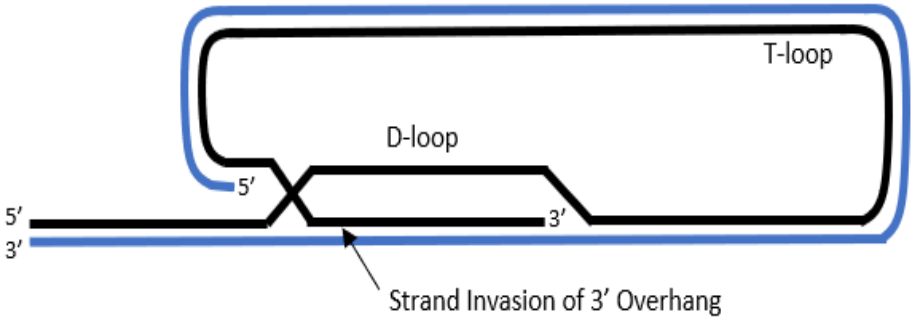

Figure 7. Structure of the telomeric T-loop and the secondary D-loop. Adapted from Strachan et al., 2015.

The Shelterin Complex

As previously described, GQ structures and T-loops work in conjunction with a group of telomere-specific proteins to regulate TL and perform the telomeric capping function. These telomere-specific proteins are collectively referred to as the shelterin complex (de Lange, 2005; Diotti & Loayza, 2011). The shelterin complex is a group of six telomere-binding proteins, telomere repeat bindings factors 1 and 2 (TRF1 and TRF2, respectively), *Ras*-related protein 1 (RAP1), protection of telomeres protein 1 (POT1), TRF1-interacting nuclear factor 2 (TIN2), and tripeptidyl peptidase 1 (TPP1), that bind to the terminal ends of telomeres, thereby stabilizing the telomere end-structure and repressing DNA damage response pathways (Figure 8) (de Lange, 2006; Diotti & Loayza, 2011). Shelterin is assembled via

the binding of telomeric double-stranded DNA by homodimers of TRF1 and TRF2, which, in turn, recruit RAP1, TIN2, TPP1, and POT1 proteins (Diotti & Loayza, 2011; Xin et al., 2008) (Figure 8). Once assembled, the shelterin complex stabilizes the telomeric structure and promotes genomic stability (de Lange, 2006). Depletion or loss-of-function of any of the components of shelterin can result in the activation of DNA damage response pathways [e.g., ataxia-telangiectasia-mutated (ATM), ataxia telangiectasia and RAD3-related (ATR), non-homologous end joining (NHEJ), and homology directed repair (HDR) pathways], replicative senescence and apoptosis, and genomic instability (de Lange, 2006; Diotti & Loayza, 2011).

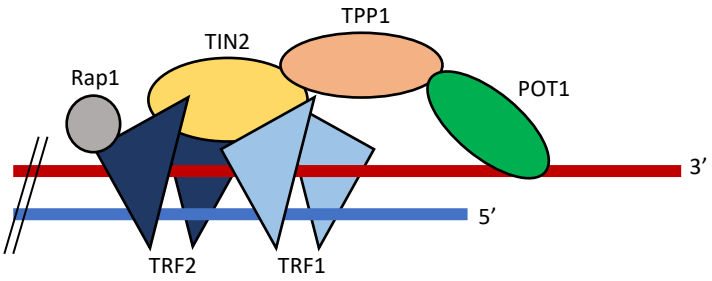

Figure 8. Structure of the shelterin complex and its association with the telomeric sequence. The shelterin complex stabilizes the telomeric end-structure and protects telomeres from degradation. Adapted from Ilicheva et al., 2015.

2.3.1.2 Telomerase and The End-Replication Problem

Replication of the ends of linear chromosomes pose a unique problem to the DNA-replication machinery (Blackburn, 1991; Wellinger, 2014). During DNA replication, conventional DNA polymerases synthesize new DNA in the 5' to 3' direction and cannot begin synthesis *de novo* (Vega et al., 2003). Instead, DNA polymerases require a short 8-12 bp RNA primer with an available 3' end (Vega et al., 2003; Wellinger, 2014). In semi-conservative replication, the leading strand is continuously synthesized, whereas the lagging strand is synthesized as short, RNA-primed Okazaki fragments (Strachan et al., 2015; Vega et al., 2003). However, due to DNA polymerases' replicative restrictions, the 3' ends of newly

synthesized lagging strands, which are minimally the length of the RNA primer, cannot be fully synthesized (i.e., the end-replication problem) (Wellinger, 2014). As a result, there is potential for the loss of important genomic information and rapid genomic instability (Blackburn, 1991; Wellinger, 2014). Consistent with this hypothesis, telomere shortening has been observed with progressive mitotic divisions (Blackburn, 1991; Blackburn et al., 1989). Indeed, 30-200 bp of telomeric DNA are lost with each cell division, with approximately 5% of this loss being the direct result of the end-replication problem (i.e., the inability of DNA polymerase to synthesize the distal ends of the telomeric sequence) (Reichert & Stier, 2017). To overcome the end-replication problem and maintain genomic stability, humans have made use of telomerase—a ribonucleoprotein enzyme that is capable of elongating the telomeric sequence (Strachan et al., 2015; Wellinger, 2014).

Telomerase is an RNA-dependent DNA polymerase (i.e., a reverse transcriptase) that synthesizes telomeric DNA sequences (Cong et al., 2002; Wellinger, 2014). This enzyme is composed of two components: telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, and telomerase RNA complex (TERC), a non-coding RNA that serves as a template for telomere elongation (Figure 9) (Strachan et al., 2015). Telomerase is expressed during the early stages of human embryogenesis, as well as in the male germline, activated lymphocytes, and stem cells (Cong et al., 2002; Strachan et al., 2015). In contrast, telomerase is either not expressed or minimally expressed in somatic cells, such as cardiac myocytes and VSMCs (Cong et al., 2002; Strachan et al., 2015). In telomere replication or elongation, telomerase is recruited to the telomeric ends by the shelterin complex (Nandakumar & Cech, 2013). At this point, TERC readily base pairs with the telomeric sequence and TERT can begin telomere synthesis (Nandakumar & Cech, 2013). Interestingly, it has been shown that telomerase preferentially acts on shortened telomeres (Baragetti et al., 2015; Sabourin et al., 2007), suggesting that structural factors, such as overall TL or telomere accessibility, may also play important roles in telomerase recruitment (Sabourin et al., 2007).

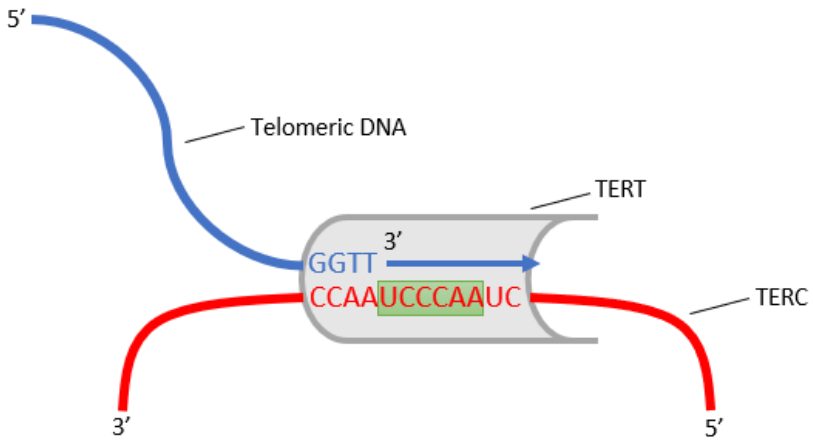

Figure 9**.** The telomerase enzyme. Telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, uses an RNA template provided by telomerase RNA complex (TERC), to replicate and elongate the telomeric sequence. Adapted from Strachan et al., 2015.

The telomeric 3’-overhang poses an additional end-replication problem for the leading strand. Leading-strand synthesis proceeds to the very end of the template strand, resulting in the production of a blunt end (Wellinger, 2014). However, this blunt end must undergo subsequent 5’-resection and fill-in synthesis in order to produce an appropriate 3’-overhang (Figure 10) (Wellinger, 2014).

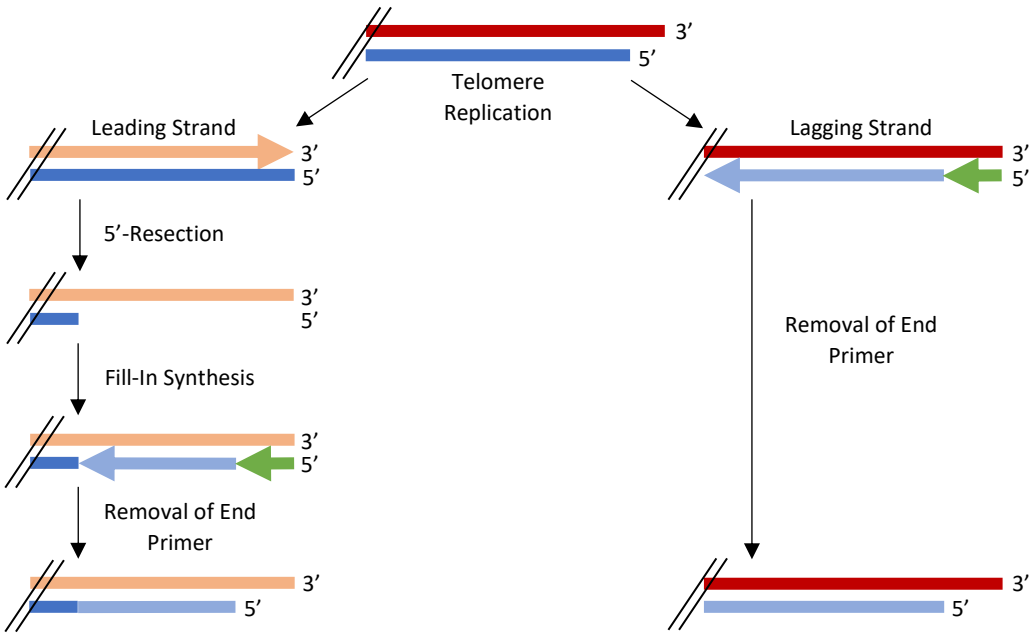

Figure 10. The end-replication problem. Dark red/blue lines show parental strands; light red/blue lines, daughter strands; green arrows, RNA primers. Lagging strand synthesis results in loss of telomeric sequence due to the removal of the end primer; leading strand synthesis requires a 5’-resection and fill-in synthesis in order to produce a 3’-overhang. Adapted from Wellinger, 2014.

2.3.1.3 Factors Affecting Telomere Length

Innate TL

Two key determinants of TL have been proposed as having major influences on disease susceptibility: innate TL and the rate of telomere attrition (Aviv et al., 2018; Entringer et al., 2018; Gorenjak et al., 2019). Innate TL, which is defined as an individual’s average basal TL, varies in humans and is largely determined by genetics (Fasching, 2018; Gorenjak et al., 2019). Indeed, TL is a highly heritable trait, with family and twin studies suggesting that 36-86% of the variation in TL at birth is explained by genetics (Fasching, 2018; Gorenjak et al., 2019). Several large genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) associated with a reduced innate TL and an increased risk of disease, with most of these SNPs affecting genes that encode for proteins involved in telomere maintenance and replication (e.g., *TERC* and *TERT*, or any of the genes

encoding shelterin proteins) (Codd, 2014; Codd et al., 2013; Fasching, 2018; Gorenjak et al., 2019). In addition to genetics, innate TL can be influenced by various other factors, including sex (Axson et al., 2018; Benetos et al., 2014), paternal age at conception (Kimura et al., 2008; Prescott et al., 2012), and antenatal exposures to various maternal stressors (Fasching, 2018; Gorenjak et al., 2019).

Several studies have revealed that females, on average, have longer TLs compared to males (Axson et al., 2018; Benetos et al., 2014; Mayer et al., 2006, 2009). Although unconfirmed, it has been postulated that hormonal differences between males and females may contribute to the observed difference in TL between sexes (Benetos et al., 2014; Gorenjak et al., 2019). Specifically, estrogen has been shown to stimulate telomerase activity, which, in turn, would increase TL in females (Benetos et al., 2014; Li et al., 2010). However, some studies have failed to demonstrate significant mediating effects of sex hormones on TL (Axson et al., 2018). In addition to sex differences, paternal age at conception has also been associated with TL (Factor-litvak et al., 2016; Kimura et al., 2008; Prescott et al., 2012). Indeed, it has been demonstrated that children with older fathers have longer telomeres, on average, than their peers with younger fathers, with a 1-year increase in paternal age at conception being associated with a 0.016 kb increase in TL at birth (Factor-litvak et al., 2016)—a phenomenon that has been attributed to heightened telomerase activity in the sperm of older males (Gorenjak et al., 2019; Kimura et al., 2008).

Antenatal exposures to maternal stressors have also been associated with innate TL. Maternal obesity during pregnancy can have adverse effects on newborn TL (Martens et al., 2016). For example, high maternal BMI prior to pregnancy has been associated with shortened innate TL, independent of parental age, gestational age, sex, birthweight, maternal smoking status, and mode of delivery (Martens et al., 2016; McCloskey et al., 2018). Similarly, maternal smoking during pregnancy has been linked to shortened innate TL (Mirzakhani et al., 2017; Salihu et al., 2015). It has been postulated that an increased burden of oxidative stress and inflammation mediate the association between these antenatal exposures and innate TL (Gorenjak et al., 2019; McCloskey et al., 2018). Finally, and perhaps the most

intriguing, is the reported association between maternal psychosocial stress during pregnancy and reduced TL in the newborn (Entringer et al., 2012). Entringer et al. (2011) were the first to show that antenatal exposures to maternal psychosocial stress were associated with reduced TL in their offspring in young adulthood (Entringer et al., 2011). This finding was replicated when Entringer et al. (2013) demonstrated that maternal psychosocial stress exposure was associated with reduced newborn TL, independent of maternal age, weight, sex, and antepartum complications (Entringer et al., 2014). Furthermore, this study concluded that the effect of pregnancy-specific stress accounted for up to 25% of the variance in TL at birth (Entringer et al., 2014, 2018). It has been postulated that maternal stress during fetal development may result in dysfunctional metabolic and inflammatory responses during gestation (Fasching, 2018; Gorenjak et al., 2019), resulting in a greater oxidative and inflammatory burden, which are known to reduce TL (Entringer et al., 2012). Thus, maternal psychosocial stress may exert a "programming" effect on innate TL, which may ultimately predispose an individual to early biological aging and an increased risk of disease (Entringer et al., 2018).

Telomere Attrition Rate

In addition to factors affecting innate TL, factors that affect telomere attrition rate may also influence biological aging and disease susceptibility (Aviv et al., 2018; Entringer et al., 2018). Of these factors, aging is considered the main determinant of telomere attrition rate over the life course (Blackburn, 1991; Gorenjak et al., 2019). Indeed, the telomere attrition rate is not stable throughout life, with studies by both Frenck et al. (1998) and Zeichner et al. (1999) revealing that the rate of attrition occurs in two characteristic phases (Frenck et al., 1998; Zeichner et al., 1999). The first phase occurs during the first 3 years of life, at which time mean TL rapidly declines, which is likely due to the high rate of cellular proliferation occurring during this growth period (Frenck et al., 1998; Zeichner et al., 1999). In the second phase, which occurs after the age of 3, the rate of TL attrition is relatively constant at a rate of approximately 50-70 bp per year (Frenck et al., 1998; Whittemore et al., 2019; Zeichner et al., 1999).

Several other factors have also been demonstrated to influence telomere attrition rate (Fasching, 2018; Gorenjak et al., 2019). For example, cigarette smoking has been associated with a reduction in TL, with two recent meta-analyses revealing that mean TL is shorter in smokers compared to non-smokers (Astuti et al., 2017; Bateson et al., 2019). Additionally, Bateson et al. (2019) found an approximately 85-bp difference in TL between smokers and non-smokers (Bateson et al., 2019), and Latifovic et al. (2016) found that even one pack-year smoked resulted in a significant reduction in mean TL (Latifovic et al., 2016). It was also noted that the association between smoking and TL appears to be influenced by smoking intensity, with Astuti et al. (2017) demonstrating a significant inverse dose-response relationship between pack-years smoked and mean TL (Astuti et al., 2017).

Similarly, obesity has been shown to be inversely associated with TL over the life course (Lee et al., 2011; Mundstock et al., 2015; Valdes et al., 2005). For example, the Fels Longitudinal Study (2011) demonstrated a significant inverse association between mean TL and BMI in a sample of healthy children and adults, independent of age and sex (Lee et al., 2011). Buxton et al. (2011) also demonstrated an independent inverse association between early-onset obesity (BMI > 90^{th} age- and sex-specific percentile) and mean TL in a large case-control study of French children (Buxton et al., 2011). Additionally, this study revealed that mean TL is nearly 24% shorter in obese children compared to non-obese children (Buxton et al., 2011). It is likely that smoking- and obesity-associated oxidative stress and inflammation mediate the association between telomere attrition rate and smoking and obesity (Lee et al., 2011; Welendorf et al., 2019).

Finally, stressful life events have been linked to reduced TL (Epel et al., 2004). In one of the first studies to examine TL in the context of psychosocial stress, Epel et al. (2004) found that TL of women aged 20-50 years exposed to high levels of perceived or chronic stress were, on average, 550-bp shorter than those of women exposed to low levels of stress, independent of age (Epel et al., 2004). Based off estimates of an approximate 50-70 bp reduction in mean TL per year, this 550-bp difference roughly

translates to an 8-11 year difference in biological age, irrespective of chronological age (Epel et al., 2004; Whittemore et al., 2019). This finding that life stress can accelerate telomere attrition has been replicated on several occasions (Mathur et al., 2017), and has even been extended to stresses occurring in childhood, such as ACEs (Bürgin et al., 2019; Coimbra et al., 2017). Indeed, early adversity, childhood trauma, and childhood psychosocial stressors have been associated with reduced TL over the life course, suggesting that early adversity may biologically embed itself and potentially contribute to an increased risk of disease (Hanssen et al., 2017; Li et al., 2017).

Tyrka et al. (2011) were the first to provide evidence of an association between childhood maltreatment and reduced TL in a sample (*n* = 31) of healthy adults aged 18-64 years (Tyrka et al., 2011). In this study, individuals who had been exposed to at least one ACE (i.e., emotional, physical, or sexual abuse or neglect) had significantly shorter TLs compared to those who had no ACEs exposures**,** independent of age, sex, BMI, smoking status, and any pre-existing medical condition (Tyrka et al., 2011). This same group of researchers then replicated this finding when they demonstrated significantly shorter TLs in those exposed to childhood adversity, independent of age, sex, and BMI, in a much larger sample (*n* = 290) (Tyrka et al., 2017). Several other studies have also demonstrated significantly reduced TLs in adults exposed to childhood adversity (Cai et al., 2015; Kananen et al., 2010; Kiecolt-Glaser et al., 2012; McFarland et al., 2018; Osler et al., 2016; Puterman et al., 2016; Surtees et al., 2011; Zalli et al., 2014). For example, Kiecolt-Glaser et al. (2012) found that TL of those exposed to two or more ACEs were on average 640-bp shorter than those without any ACEs exposures—a difference that roughly translated to a 9-13 year difference in biological age, irrespective of chronological age (Kiecolt-Glaser et al., 2012). Moreover, this difference in TL could not be explained by age, sex, or BMI (Kiecolt-Glaser et al., 2012). Additionally, both Surtees et al. (2011) and Puterman et al. (2016) found evidence of a dose-response relationship between childhood adversity and TL, such that TL was reduced for each additional ACE exposure (Puterman et al., 2016; Surtees et al., 2011).

Studies have also examined the effects of childhood adversity in child populations (Drury et al., 2014; Mitchell et al., 2010; Shalev et al., 2013). Indeed, Drury et al. (2014) reported significantly shorter TLs in children aged 5-15 years who were exposed to household dysfunction (i.e., family violence, family suicide, or incarceration) (Drury et al., 2014). Similarly, Mitchell et al. (2014) found that an exposure to household dysfunction was associated with a 40% reduction in TL in a sample of boys aged 9-10 years, independent of parental age at birth, mother's education, and BMI (Mitchell et al., 2014). Finally, in a longitudinal study examining the association between exposure to violence and telomere attrition in children, Shalev et al. (2013) demonstrated that children with two or more exposures to violence (i.e., domestic violence between mother and partner, frequent bullying victimization, or physical maltreatment by an adult) had significantly increased rates of telomere attrition (i.e., shorter TLs) compared to their non-exposed peers, independent of sex and BMI (Shalev et al., 2013). Additionally, they found that physical maltreatment alone was significantly and independently associated with an increased rate of TL attrition (Shalev et al., 2013).

Importantly, not all studies have demonstrated an association between childhood adversity and TL in adulthood (Glass, 2010; Jodczyk et al., 2014; van Ockenburg et al., 2015a; Verhoeven et al., 2015). Glass et al. (2010) reported that there was no significant difference in TL between individuals exposed to physical or sexual abuse and those not exposed to abuse (Glass, 2010). Similarly, both Verhoeven et al. (2015) and van Ockenburg et al. (2015) showed that TL in adulthood (aged 18-65 and 33-79 years, respectively) was not associated with any form of childhood adversity examined (i.e., abuse, neglect, household dysfunction) (van Ockenburg et al., 2015; Verhoeven et al., 2015). However, it should be noted that the latter two studies found significantly shorter TLs in those exposed to recent stressful life events (within the past 5 years) (van Ockenburg et al., 2015a; Verhoeven et al., 2015). Despite these null findings and the between-study heterogeneity that exists within the literature, recent systematic

reviews and meta-analyses suggest that ACEs are associated with a reduction in TL, albeit with a small effect size (Bürgin et al., 2019; Hanssen et al., 2017; Li et al., 2017; Ridout et al., 2018).

2.3.1.4 Telomere Length and Cardiovascular Disease

Finally, shorter TLs have been associated with an increased risk of CVD, independent of traditional CV risk factors (Haycock et al., 2014). Indeed, a large meta-analysis (*n* = 24) revealed that, in comparison of the shortest versus longest third of TL, those in the shortest third of TL were at a 1.54-fold increased risk of CHD (Haycock et al., 2014), a finding that was replicated by Xu et al. (2019), who not only found shorter TLs in those with CHD, but also found that TL was inversely associated with the severity of CHD (Xu et al., 2019). In addition, prior research has demonstrated significant associations between shorter TL and MI, congestive heart failure, atherosclerotic CVD, hypertension, and overall CVD risk (De Meyer et al., 2018; Rehkopf et al., 2016), as well as various CVD risk factors, including obesity, blood lipids, inflammatory biomarkers, and measures of arterial stiffness (Benetos et al., 2001; McDonnell et al., 2017; Rehkopf et al., 2016). For example, Benetos et al. (2001) found that shorter TL was associated with a faster cfPWV, independent of MAP and age, in a population of males (mean age 55.0 ± 1.0 years) (Benetos et al., 2001). Interestingly, however, the authors found no such association in females of the same age stratum, which may have been due to the small sample size of females in their study or to potential differences in the progression of arterial stiffness between sexes (Benetos et al., 2001). Wang et al. (2011) replicated this finding when they demonstrated a significant inverse association between cfPWV and TL in older males aged (mean age 57.7 ± 11.6 years), independent of age (Wang et al., 2011). In comparison, both Strazhesko et al. (2015) and McDonnell et al. (2017) found that cfPWV was independently and inversely associated with TL in both males and females (McDonnell et al., 2017; Strazhesko et al., 2015). Interestingly, McDonnell et al. revealed a significant modifying effect of age on the association between cfPWV and TL, in which cfPWV was significantly inversely associated with TL in younger individuals (< 30 years), but positively associated with TL in older

individuals (> 50 years) (McDonnell et al., 2017). However, it should be noted that the positive association observed in older individuals may have been the result of a healthy survivor effect; that is, individuals with high cfPWV and short TL may have been selected out of the analysis because of the presence or a history of CVD (McDonnell et al., 2017). It is unclear whether telomere shortening represents a causative biomarker in the progression of arterial stiffness or rather an epiphenomenon arising from shared underlying biological stress. Despite this, these studies provide preliminary evidence that TL—as an index of biological stress—may be associated with central artery stiffness.

2.3.1.5 Telomere Length Measurement

Several methods have been developed for the measurement of TL (Cawthon, 2002, 2009; Kimura et al., 2010; Montpetit et al., 2015; O'Callaghan & Fenech, 2011). Terminal restriction fragment (TRF) analysis was the original technique developed for determining mean TL and is currently regarded as the gold-standard (Aubert et al., 2012; Lai et al., 2018; Montpetit et al., 2015). TRF analysis exploits the specific and repetitive nature of the telomeric sequence by digesting genomic DNA with a combination of restriction enzymes, which specifically exclude telomeric repeats, resulting in short genomic fragments and longer undigested telomeres (Aubert et al., 2012; Montpetit et al., 2015). The intact telomeres are then resolved based on size by agarose gel electrophoresis and telomere fragments are detected through either Southern blotting or in-gel hybridization using a labeled probe specific to the telomeric sequence (Aubert et al., 2012; Montpetit et al., 2015). The varying lengths of telomeres will present as a smear, which can then be compared to a standard DNA ladder of known fragment sizes to estimate mean TL (Aubert et al., 2012; Kimura et al., 2010).

Although this method is considered the gold-standard, it is still subject to a number of limitations (Lai et al., 2018; Montpetit et al., 2015). For example, the use of an intact DNA sample is integral to the application of this technique, as any DNA degradation could lead to inaccuracies in TL measurements, producing a bias toward shorter TLs (Montpetit et al., 2015). Similarly, the restriction

enzymes used may result in the inclusion of sub-telomeric DNA that is contiguous to the telomeric sequence, thereby leading to an overestimation of mean TL (Lai et al., 2018; Montpetit et al., 2015). Another limitation of this technique is that there are no standardized combinations of restriction enzymes used in TRF analysis, making comparisons in TL between different studies difficult (Aubert et al., 2012). TRF analysis also requires a significant amount of DNA (i.e., micrograms), rendering it more applicable to analyzing TL in blood samples rather than other tissue samples (e.g., saliva) (Aubert et al., 2012; Lai et al., 2018; Montpetit et al., 2015). Finally, this technique tends to be very laborious, and it cannot be used to detect very short telomeres due to hybridization limitations (i.e., inability of probe to bind shortest telomeres) (Aubert et al., 2012; Lai et al., 2018; Montpetit et al., 2015).

To overcome the need for large quantities of DNA to evaluate TL, quantitative polymerase chain reaction (qPCR)-based methods of determining TL have been developed (Cawthon, 2002, 2009; O'Callaghan & Fenech, 2011). qPCR is the most frequently used method of estimating TL (Cawthon, 2002; Montpetit et al., 2015). This method allows for the detection and quantification of a specific DNA segment from a complex mixture of DNA, and relies heavily on the use of heat-stable DNA polymerases and specific oligonucleotide primers to amplify the segment of interest via thermal cycling procedures (Garibyan & Avashia, 2014; Strachan et al., 2015). Unlike TRF analysis, qPCR-based methods for TL quantification require much less DNA (e.g., nanograms) and can be used on samples that have been slightly degraded thus, making qPCR applicable to various source tissues (e.g., blood, saliva, etc.) (Montpetit et al., 2015; Strachan et al., 2015). Furthermore, qPCR-based methods are low-cost and less laborious relative to TRF analysis, making them better suited for large epidemiological studies (Lai et al., 2018; Montpetit et al., 2015).

In addition to TRF analysis and PCR-based TL measurements, several other methods have been developed to quantify TL. Of note, single telomere length analysis, quantitative fluorescence in situ hybridization, and telomere shortest length assay have been used to quantify TL; however, due to their

lack of utility and use in common practice, they will not be discussed in detail (see Aubert et al., 2012, Montpetit et al., 2015, and Lai et al., 2018 for a detailed review).

2.3.2 Mitochondria

Similar to telomeres, mitochondria may be particularly susceptible to biological stress and thus, may provide a link between ACEs and CVD (Ridout et al., 2018). Mitochondria are dynamic organelles that produce energy in the form of ATP (the primary energy source of cells), but also play vital roles in intracellular signaling, lipid and nucleic acid biosynthesis, calcium homeostasis, and ROS production (O'Hara et al., 2019; Ridout et al., 2018). In contrast to other organelles, mitochondria house their own genome essential for mitochondrial bioenergetics (Ridout et al., 2018; Strachan et al., 2015). This genome consists of a circular, double-stranded 16.6-kb mitochondrial DNA (mtDNA) that encodes for protein components essential to oxidative phosphorylation and mitochondrial protein synthesis (Strachan et al., 2015; Yue et al., 2018). MtDNA is highly susceptible to oxidative damage and mutations due to its continual exposure to mitochondrial ROS, lack of histone proteins, and limited DNA damage repair capacity (Cadenas & Davies, 2000; Ridout et al., 2018; Yakes & Van Houten, 1997). As such, the extent of mtDNA damage may be reflected in copy number (mtDNAcn) (Yue et al., 2018). Unlike the nuclear genome that exists in two copies per diploid cell, various cells may contain hundreds to thousands of copies of mtDNA (O'Hara et al., 2019; Strachan et al., 2015). The number of mtDNA copies per cell (i.e., mtDNAcn) varies across cell type depending on energy demands (Strachan et al., 2015), and is heavily influenced by biological stress (O'Hara et al., 2019). Once mtDNA damage occurs, mtDNAcn is altered, and mitochondria become dysfunctional (Yue et al., 2018). Mitochondrial dysfunction and lower mtDNAcn have been associated with several CVDs (Yue et al., 2018), including CHD (Liu et al., 2017) and heart failure (Huang et al., 2016). Moreover, mitochondrial dysfunction and mtDNAcn have been associated with central artery stiffness in mice, but not humans (Foote et al., 2018; LaRocca et al., 2014;

Zhou et al., 2012). Recent studies have also found a significant positive association between childhood adversity and mtDNAcn (Ridout et al., 2019; Tyrka et al., 2016).

2.3.2.1 Structure and Function

Mitochondria are unique double membrane-bound organelles that appear as ovoid or rod-shaped particles within the cytoplasm (Cowan et al., 2016). These organelles vary in length (1-10 μm), have near constant diameter (approximately 700 nm) (Figure 11) (McCarron et al., 2013), and often exist as a system of budding and fusing networks (Chinnery, 2003). Both the inner and outer mitochondrial membranes are composed of several classes of phospholipids that promote bilayer and non-bilayer regions (Basu Ball et al., 2018). The outer mitochondrial membrane forms a smooth, continuous surface that surrounds the entire organelle and forms the external contour, whereas the inner mitochondrial membrane contains numerous conically-shaped phospholipids that promote the formation of negative curvatures (i.e., folds) referred to as cristae (Figure 11) (Basu Ball et al., 2018; Cowan et al., 2016). Additionally, the outer membrane contains a number of porins that permit the diffusion of molecules into the intermembrane space, whereas the inner membrane is largely impermeable (McCarron et al., 2013). The cristae of mitochondria provide a large surface area for the organization of the electron transport chain (ETC), which is essential for oxidative phosphorylation and ultimately ATP synthesis (Basu Ball et al., 2018). Deep to the cristae lies the mitochondrial matrix, which contains ribosomes, mtDNA, proteins of the citric acid cycle, and various matrix granules (Figure 11) (Cowan et al., 2016). The main function of mitochondria is to produce energy via the breakdown of organic molecules (e.g., glucose and fatty acids) and cellular respiration; however, they also play critical roles in various other cellular processes (O'Hara et al., 2019; Ridout et al., 2018).

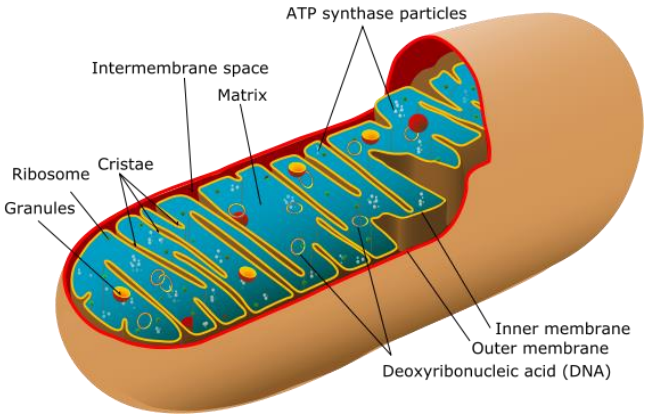

Figure 11. Anatomy of mitochondria.
Used with permission: Mariana Ruiz Villarreal. (2006). "Animal Mitochondrion Diagram''. *Wikimedia Commons.* *https://commons.wikimedia.org/wiki/File:Animal_mitochondrion_diagram_en.svg*

2.3.2.2 Mitochondrial DNA

As previously described, human mtDNA is a double-stranded, circular 16.6-kb genome that closely resembles that of a reduced, or stripped-down, bacterial genome (Figure 12) (Strachan et al., 2015). MtDNA resides within the mitochondrial matrix and is composed of a heavy and light strand, which collectively contain 37 genes (Chocron et al., 2019). Of these genes, 13 encode protein components of the ETC essential to oxidative phosphorylation, and the remaining genes encode 22 tRNAs and 2 rRNAs that are essential to protein synthesis about the mitochondrial ribosomes (Strachan et al., 2015). The mitochondrial genome also contains a fairly large non-coding region of about 1-kb that contains regulatory elements for the initiation and termination of mtDNA transcription (Chocron et al., 2019). Within this non-coding region, mtDNA also contains a D-loop, which contains the heavy strand origin of replication (OriH) (Chocron et al., 2019). Furthermore, mtDNA is not naked, but instead packaged into a nucleoid structure with a group of protein factors that each contain roughly 5-7 copies of the mitochondrial genome (Montier et al., 2009). These nucleoid-associated proteins, which include the mitochondrial transcription factor A (TFAM), mitochondrial single-strand binding protein, and the

helicase Twinkle, are essential for mtDNA replication and protein synthesis (Montier et al., 2009; Foote et al., 2018).

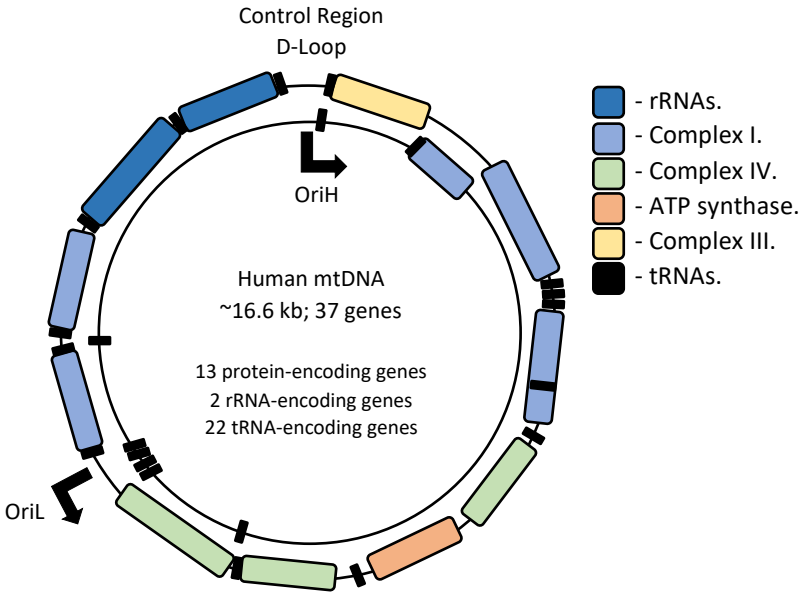

Figure 12. Anatomy of human mitochondrial DNA (mtDNA). OriH is the heavy strand origin of replication and OriL is the light strand origin of replication. Adapted from Chocron et al., 2019.

Importantly, the mitochondrial genome does not encode for all of the proteins necessary for mitochondrial function. Instead, the additional protein components required for oxidative phosphorylation, and other required mitochondrial proteins, are encoded within the nuclear genome and are subsequently transported into mitochondria (Strachan et al., 2015). In contrast to the nuclear genome, the mitochondrial genome is a model of “economical” DNA usage (Strachan et al., 2015). Indeed, more than 90% of the mtDNA sequence directly encodes for proteins or functional non-coding RNA (i.e., rRNAs and tRNAs), and no mitochondrial gene is interrupted by an intron (Strachan et al., 2015). In comparison, the nuclear genome, which is large (approximately 3.2-Gb) and complex, has a much lower gene density and genes are frequently interrupted by introns (Strachan et al., 2015). Additionally, the nuclear genome contains large amounts of repetitive non-coding sequence (e.g., telomeres), whereas the mitochondrial genome does not (Strachan et al., 2015).

Mitochondrial DNA Copy Number

In comparison to nuclear DNA, which exists in two copies per diploid cell, the number of mtDNA copies per diploid cell can vary from hundreds to thousands depending on cell type (i.e., energy demands), and is heavily influenced by biological stress (O'Hara et al., 2019). For example, mitochondria account for 40% of the cardiac cell volume, and 90% of the energy required for the heart is supplied by mitochondria (Yue et al., 2018). Accordingly, mtDNAcn in cardiac cells has been shown to be greater than 6000 copies per cell (6970 ± 920) (Miller, 2003). MtDNA is also particularly susceptible to oxidative damage and mutations due to its continual exposure to mitochondrial ROS, lack of histone proteins, and limited DNA damage repair capacity (Cadenas & Davies, 2000; Ridout et al., 2018; Yakes & Van Houten, 1997). As such, the extent of mtDNA damage may be reflected in mtDNAcn, which has been shown to be lower in several diseases, including CVD (Clay Montier et al., 2009; Yue et al., 2018).

Finally, in comparison to the abundance of factors associated with TL and telomere attrition rate, relatively less is known about factors affecting mtDNAcn outside of oxidative stress and inflammation (Liu et al., 2003; Wu et al., 2017). In the CARDIA Study, Révész et al. (2018) revealed that mtDNAcn decreased with age, obesity, and high blood glucose and lipid concentrations over a 10-year period in a population of US adults (mean age 50.4 ± 3.6 years at follow-up) (Révész et al., 2018). Furthermore, the authors found that an increasing number of metabolic dysregulations at baseline (i.e., abdominal obesity, hypertriglyceridemia, low HDL cholesterol, hyperglycemia, and hypertension) were associated with a reduction in mtDNAcn at 10-year follow-up, which the authors suggested may be the result of an increasing oxidative and inflammatory load (Révész et al., 2018). Interestingly, Revesz et al. also replicated a previous finding that mtDNAcn was positively associated with TL (Révész et al., 2018; Tyrka et al., 2015), suggesting that telomeres and mitochondria may be functionally linked (Tyrka et al., 2015). In another study, Brunst et al. (2017) found that maternal lifetime psychosocial stress and maternal antenatal stress were independently associated with a reduction in placental mtDNAcn (Brunst

et al., 2017). These authors also concluded that the oxidative and inflammatory burden associated with maternal psychosocial stress may reduce placental mtDNAcn and ultimately influence mitochondrial function in their offspring (Brunst et al., 2017). Finally, two studies have revealed associations between ACEs and mtDNAcn (Ridout et al., 2019; Tyrka et al., 2016). In the first of these studies, Tyrka et al. (2016) found that those exposed to childhood maltreatment had a significantly higher mtDNAcn compared to those who were not exposed to childhood maltreatment, independent of age, sex, BMI, education, and childhood SES, in a sample of healthy adults (*n* = 290; mean age 31.0 ± 10.7 years) (Tyrka et al., 2016). Similarly, Ridout et al. (2019) found a significant positive association between mtDNAcn and childhood adversity in a sample of preschool-aged children (*n* = 256; mean age 4.3 ± 0.7 years) with substantiated cases of childhood maltreatment, independent of age and ethnicity (Ridout et al., 2019). Together, these studies provide preliminary evidence of the potential early effects of ACEs on mitochondrial function.

2.3.2.3 Mitochondrial DNA Copy Number and Cardiovascular Disease

As previously described, mitochondria play a critical role in energy homeostasis, which is largely dependent on proteins translated from genes encoded by mtDNA. Mitochondrial dysfunction, a hallmark of biological aging, disrupts energy homeostasis and is believed to be a core component of several chronic diseases, including CVD (Dai et al., 2012). Indeed, mitochondrial dysfunction may be critical in the development and progression of atherosclerosis (Madamanchi & Runge, 2007; Peng et al., 2019) and heart failure (Zhou & Tian, 2018); however, less is known about the role of mitochondrial dysfunction in central artery stiffness. Although not a direct measure of mtDNA damage or mitochondrial dysfunction, mtDNAcn is associated with mitochondrial respiration (Foote et al., 2018; Jeng et al., 2008), and may therefore serve as a biomarker of mitochondrial function (Ashar et al., 2017; Yue et al., 2018). Recently, several studies have examined the association between mtDNAcn and adverse CV health outcomes, including heart failure (Huang et al., 2016), CHD (Chen et al., 2014; Liu et

al., 2017), and sudden cardiac death (Zhang et al., 2017). These studies consistently demonstrated a reduction in mtDNAcn in those with CVD (Chen et al., 2014; Huang et al., 2016; Liu et al., 2017; Zhang et al., 2017). Furthermore, a meta-analysis comprised of three large, population-based prospective studies (*n* > 11,900; mean age 62.4 years) found that a 1-SD decrease in mtDNAcn was independently associated with a 1.29-fold increased risk of CHD, a 1.11-fold increased risk of stroke, and a 1.23-fold increased risk of any CVD (Ashar et al., 2017). Importantly, the aforementioned studies examined the association between mtDNAcn and CV health in middle-aged and older adults (> 45 years), and none examined the association between mtDNAcn and central artery stiffness.

Recent murine models have also revealed an importance of mitochondrial function in central artery stiffness (Foote et al., 2018; Gioscia-Ryan et al., 2018; LaRocca et al., 2014). For example, Foote et al. (2018) showed that a reduction in mtDNAcn was associated with a reduction in mitochondrial respiration, and a greater degree of CCA and aortic stiffness in mice (Foote et al., 2018). The authors also found that restoring mtDNAcn improved both mitochondrial respiration and arterial elasticity (Foote et al., 2018). In another study, LaRocca et al. (2014) found that mitochondrial dysfunction was associated with a faster cfPWV in aging mice (LaRocca et al., 2014). Similar to the study by Foote et al., the researchers found that restoring mitochondrial function through TFAM overexpression preserved aortic elasticity (LaRocca et al., 2014). Finally, Gioscia-Ryan et al. (2018) found that treating aged mice with a mitochondria-targeted antioxidant (i.e., MitoQ) reduced their level of aortic stiffness (Gioscia-Ryan et al., 2018). Interestingly, the authors also found that antioxidant therapy partially attenuated elastin turnover within the aortic wall ECM (Gioscia-Ryan et al., 2018). As a result, it was concluded that mitochondrial oxidative stress may exert detrimental effects on central artery stiffness via the degradation of elastin (Gioscia-Ryan et al., 2018). Although not examined in humans, the results of the aforementioned studies provide some evidence that mitochondrial dysfunction, as indexed by reduced mitochondrial respiration and mtDNAcn, and increased ROS production, may play a role in the

progression of central artery stiffness. Moreover, the study by Foote et al. provides preliminary support for the hypothesis that mtDNAcn may be reduced in individuals with greater arterial stiffness.

2.3.2.4 Association between Mitochondria and Telomeres

As previously described, mitochondria are the main site of ATP synthesis; however, they are also the main site of ROS production (Zheng et al., 2019). A significant portion of the electrons that pass through the ETC leak to form superoxide (Raha & Robinson, 2000), and although superoxide is relatively unreactive, it is the precursor of several damaging ROS (Raha & Robinson, 2000). Mitochondrial dysfunction-associated increases in ROS can induce oxidative stress, which, in turn, may lead to the breakage of single- and double-stranded DNA (Zheng et al., 2019a). Importantly, telomeres are particularly susceptible to oxidative damage, specifically 8-oxoG formation, due to their G-rich nature, suggesting that mitochondrial dysfunction may in fact induce telomere damage and replicative senescence (Oikawa & Kawanishi, 1999; Zheng et al., 2019a). However, the opposite may also hold true, as telomere dysfunction has been postulated to contribute to mitochondrial compromise and age-related pathology (Sahin et al., 2011; Sahin & DePinho, 2012). Telomere dysfunction activates p53-mediated cellular growth arrest, replicative senescence, and apoptosis, which lead to a progressive loss of tissue function (Lin et al., 2005; Sahin et al., 2011). Interestingly, activated p53 has also been shown to bind the promoter regions of peroxisome proliferator-activated receptor-gamma coactivator 1α and 1β (*PGC-1α* and *PGC-1β*, respectively) (Sahin et al., 2011). *PGC-1α* and *PGC-1β* are considered the master mitochondrial regulators as they regulate mitochondrial biogenesis and respiration and thus, play a critical role in energy homeostasis (Lin et al., 2005). Binding of the *PGC-1α* and *PGC-1β* promoters by activated p53 has been shown to down-regulate their expression, resulting in mitochondrial dysfunction, evidenced by compromised mitochondrial respiration (Sahin et al., 2011). Additionally, activation of p21 via telomeric sequence damage may also contribute to mitochondrial dysfunction, as activation of p21 has been associated with increased mitochondrial ROS production and mitochondrial

dysfunction via a downstream signaling cascade (Passos et al., 2010). Finally, TERT has been shown to have both telomeric and mitochondrial functions (Zheng et al., 2019a). Indeed, under conditions of oxidative stress, TERT has been shown to be exported out of the nucleus and imported into the mitochondrial matrix, where it may serve to protect mtDNA against oxidative damage; however, the exact function of mitochondrion-localized TERT remains unclear (Zheng et al., 2019).

2.3.2.5 Mitochondrial DNA Copy Number Measurement

Similar to the measurement of TL, mtDNAcn is typically assessed using one of two methods—Southern blot hybridization or qPCR techniques (Andreu et al., 2009). Southern blot analysis is commonly used to assess alterations in mtDNAcn, particularly when a mitochondrial disease is present (Andreu et al., 2009; Bai & Wong, 2005). Briefly, this method begins with the digestion of whole cell DNA by specific restriction enzymes, resulting in a collection of DNA fragments, which includes both nuclear and mtDNA fragments (Shmookler et al., 1983). The fragments are then separated according to size by gel electrophoresis and transferred to a nylon membrane (Strachan et al., 2015). A labelled probe specific to a conserved region of mtDNA can then be added to the membrane and allowed to bind, and mtDNAcn can then be determined autoradiographically in reference to the total amount of initial DNA used (Shmookler Reis & Goldstein, 1983; Strachan et al., 2015). This method, however, requires large amounts of DNA and is quite laborious (Andreu et al., 2009; Memon et al., 2017). As a result, most studies that aim to quantify mtDNAcn utilize qPCR techniques, which is currently considered the gold-standard (O'Hara et al., 2019; Refinetti et al., 2017). This method allows for the detection and quantification of a specific mtDNA segment from a complex mixture of DNA, and relies heavily on the use of heat-stable DNA polymerases and specific mtDNA primers to amplify the segment of interest via thermal cycling procedures (Garibyan & Avashia, 2014; Strachan et al., 2015). Unlike Southern blot analysis, qPCR-based methods for mtDNAcn quantification require much less DNA and can successfully amplify mtDNA fragments that have been slightly degraded (Montpetit et al., 2015; Strachan et al.,

2015). Furthermore, this method is relatively low-cost and less laborious relative to Southern blotting (Montpetit et al., 2015; Strachan et al., 2015). Thus, qPCR-based methods may be better suited for large epidemiological studies (Lai et al., 2018; Montpetit et al., 2015).

2.4 Adverse Childhood Experiences, Biological Aging, and Cardiovascular Risk

Exposures to childhood adversity have been shown to have lasting impacts on CV health well into adulthood (Danese et al., 2009; Dong et al., 2004). One mechanism by which ACEs may influence CV health is through the dysregulation of biological systems, particularly the stress response systems (Danese & McEwen, 2012; Horn et al., 2019). Dysregulation of these systems can result in oxidative stress and chronic low-grade inflammation (i.e., biological stress), which may be reflected in an individual's TL and/or mtDNAcn (Ridout et al., 2019; Tyrka et al., 2015). Telomere and mitochondrial dysfunction have been implicated in the process of biological aging (Sahin et al., 2011; Sahin & DePinho, 2012) and may reflect one mechanism by which ACEs contribute to deleterious CV health outcomes. Vascular aging (an important feature of biological aging) is characterized by a marked increase in central artery stiffness (Laurent et al., 2019; Nilsson, 2008), which has been shown to be an independent predictor of both CV morbidity and all-cause mortality (Laurent et al., 2006; Van Bortel et al., 2012). Furthermore, central artery stiffness has been observed in individuals as early as young adulthood (Wildman et al., 2003), and measures of both systemic and peripheral arterial stiffness have been observed in individuals exposed to ACEs (Klassen et al., 2016; Su et al., 2014). To summarize, ACE exposures may result in biological dysfunction, which, in turn, may be reflected by a reduction in TL and/or mtDNAcn (Figure 13). As a result of this biological dysfunction, individuals exposed to ACEs may present with a vascular phenotype characterized by a marked increase in central artery stiffness, and therefore, may be at an increased risk of deleterious CV health outcomes (Figure 13).

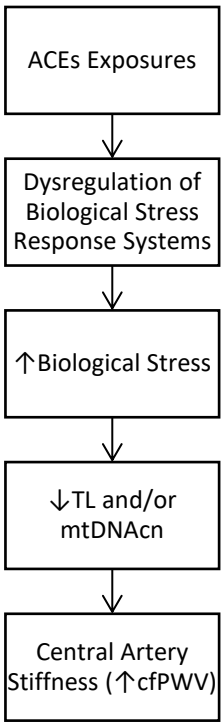

Figure 13. Proposed pathway linking ACEs with reduced TL and mtDNAcn, and ultimately increased central artery stiffness (cfPWV).

2.5 Restatement of Study Rationale and Hypotheses

Few studies have examined the association between ACEs and arterial stiffness, and none have examined the association between ACEs and central artery stiffness. Furthermore, the mechanisms by which ACEs may contribute to arterial stiffness have not yet been fully elucidated. Finally, markers of biological stress (i.e., TL and mtDNAcn) have been independently associated with both ACEs and central artery stiffness; however, several questions have yet to be answered. First, although several studies have examined the association between TL and ACEs, only two studies have explicitly examined the association between mtDNAcn and ACEs. These studies both reported a positive association between ACEs and mtDNAcn (Ridout et al., 2019; Tyrka et al., 2016); however, neither study examined the long-term effects of an increasingly adverse childhood environment (i.e., the impact of an accumulation of ACEs) on mtDNAcn, which may be more consistent with the development of disease (Malik & Czajka, 2013; Siasos et al., 2018; Yue et al., 2018). Additionally, it has been postulated that mitochondria

respond differently to acute versus chronic/persistent stress, such that acute stress may result in a compensatory increase in mtDNAcn, whereas chronic/persistent stress may result in a reduction in mtDNAcn and disease (Malik & Czajka, 2013; Siasos et al., 2018; Yue et al., 2018). Accordingly, the current study has hypothesized that mtDNAcn would be reduced in those with a faster cfPWV and those who reported experiencing a greater number of ACEs, which is in direct contrast to two previous studies (Ridout et al., 2019; Tyrka et al., 2016). Second, very few studies have examined the association between TL and central artery stiffness in healthy young populations, and none have examined the association between mtDNAcn and central artery stiffness. Here, it was hypothesized that TL would be reduced in those with a faster cfPWV and those who reported experiencing a greater number of ACEs. Finally, one way in which ACEs may contribute to the progression of central artery stiffness is through inducing biological stress; however, there have been no studies that have examined the potential modulatory effects of markers of biological stress (i.e., TL and mtDNAcn) on the association between ACEs and arterial stiffness. The current study hypothesized that markers of biological stress (i.e., TL and/or mtDNAcn) would influence the association between ACEs and cfPWV. This study will serve to fill gaps in the literature pertaining to telomere and mitochondrial biology with respect to CV health and childhood adversity, as well as expand upon the body of literature focusing on the ACEs-CV health relationship.

The aim of the current study will be to test the following hypotheses:

1) Individuals who have experienced a greater number of ACEs will have a faster carotid-femoral pulse wave velocity (cfPWV) and thus, a greater degree of central artery stiffness.
2) Individuals who have experienced a greater number of ACEs will have a higher burden of biological stress presenting as either:
    a. Shorter mean TL and/or
    b. Lower mean mtDNAcn.
3) Higher burdens of biological stress (i.e., shorter mean TL and/or lower mean mtDNAcn) will be associated with a faster cfPWV.
4) The association between ACEs and central artery stiffness will be mediated by either:
    a. Mean TL and/or
    b. Mean mtDNAcn.

Exploratory analyses will also be performed to examine the potential moderating effects of sex, smoking status, BMI, and PA on the postulated associations between ACEs and cfPWV, ACEs and markers of biological stress, and markers of biological stress and cfPWV. Furthermore, the potential moderating effects of TL and mtDNAcn on the association between ACEs and cfPWV will be explored.

Chapter III: Methodology

3.1 Study Design and Participants

The current study was carried out as part of the ongoing Niagara Longitudinal Heart Study (NLHS) that is taking place in Southern Niagara, ON, Canada. The NLHS is a follow-up study that builds on three baseline studies that took place across the Niagara region from 2007 to 2013. Each baseline study varied in its collection of demographic, psychosocial, lifestyle, and biological measures; however, all three studies collected data on various CV health parameters in a subset of their respective study populations (Wade et al., 2019). At baseline, CV data were obtained on 564 individuals who ranged in age from 8-18 years. It is these individuals (now aged 19-25 years) who are currently being re-recruited for follow-up CV assessment. Due to the perceived intrusiveness in the acquisition of the femoral PP in children and adolescents, cfPWV was not measured at baseline. Additionally, data on TL and mtDNAcn were not collected at baseline. The lack of data on these parameters at baseline precluded the current study's ability to examine longitudinal changes in these parameters. For this reason, the current study will only focus on data from the follow-up time point and will be cross-sectional in nature.

Contact information was available from each of the original studies and was used to re-recruit participants for a follow-up examination at the Brock University Human Hemodynamics Laboratory. Upon scheduling a laboratory visit, participants were sent an information package containing a letter of introduction and laboratory contact information, among other items. All participants were instructed to avoid vigorous physical activity as well as alcohol and caffeine consumption for at least 12-hours prior to their laboratory visit. Additionally, participants were asked to fast for at least 4-hours prior to their laboratory visit. Upon arrival to the laboratory, a trained researcher outlined the purpose of the study, laboratory procedures, how and why the participant was recruited, and confidentiality measures. At this time, the researcher also obtained informed consent and answered any questions the participant may have had prior to CV assessment. For a more detailed overview of the NLHS, see Wade et al. (2019).

3.2 Anthropometrics and Cardiovascular Measures

3.2.1 Heart Rate and Blood Pressure

Once the participant was made aware of the study purpose and protocol, and informed consent was obtained, they were asked to void their bladder as to prevent any effect of bladder distension on BP (Fagius & Karhuvaara, 1989). They were then outfitted with an appropriately sized BP cuff in a seated position. The cuff was positioned on their right arm, which was resting at the midpoint of the sternum supported on a table. Once the cuff was properly positioned, the participant was asked to relax with their feet flat on the floor in the seated position and to remain silent for a minimum of 15 minutes. After this period of rest, six HR and BP measurements were taken at one-minute intervals using an automated oscillometric device (BpTRU Vital Signs Monitor, BPM-300, VSM, MedTech Devices, Coquitlam, BC, Canada). Of the six measurements, the first three were used to familiarize the subject with cuff pressurization and were discarded. The final three values were used to calculate average seated SBP and DBP (Coverdale et al., 2012; Fitzgibbon et al., 2012; Phillips et al., 2015). The average of the SBP and DBP were used to calculate MAP as follows:

$$MAP = DBP + \frac{1}{3}(SBP - DBP)$$

3.2.2 Anthropometrics

Standing height (cm) was measured using a stadiometer (STAT-7X, Ellard Instrumentations, Monroe, Washington, USA) and body mass (kg) was measured using a digital scale (BWB-800S, Tanita, Tokyo, Japan). Body mass index (BMI) (kg/m^2) was calculated as body mass (kg) divided by height squared (m^2). BMI was categorized as normal weight (< 25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (> 30 kg/m^2).

3.2.3 Beat-by-Beat and PWV Assessment

Once height and body mass measures were obtained, participants rested in the supine position in a quiet, dimly lit, temperature-controlled room for the duration of their CV assessment. While supine, participants were outfitted with a standard single-lead electrocardiogram (ECG) and a finger BP cuff (Nexfin, BMEYE, Amsterdam, Horton, Norway; NIBP Nano, ADInstruments, Colorado Springs, USA) on the left middle digit in order to obtain 5 minutes of continuous HR and beat-by-beat BP measurements, respectively. Continuous HR and beat-by-beat BP measurements were collected throughout the duration of their CV assessment. One-minute averages of beat-by-beat BP and HR recordings were calculated to obtain mean instantaneous BP and HR.

Measurement of cfPWV was used to assess central artery stiffness and was defined as the speed of the pulse from the left CCA to the left femoral artery, as this is the standardized probe placement for the NLHS. This method is based on the principal that pressure waves generated during LV ejection will travel with a faster velocity through stiffer arteries compared to more elastic arteries. To obtain cfPWV, local PP waveforms were collected at the left CCA and femoral arterial sites using a handheld applanation tonometer (Millar Instruments, Houston, TX, USA). cfPWV was calculated using the formula:

$$cfPWV = \frac{D_{FA} - D_{CCA}}{T_{FA} - T_{CCA}}$$

where D_{FA} represents the sum of the distance from the suprasternal notch to the umbilicus and the umbilicus to the femoral artery, D_{CCA} represents the distance from the suprasternal notch to the CCA, T_{FA} represents the PTT to the femoral artery, and T_{CCA} represents the PTT to the CCA. Distance measurements were obtained using an inelastic tape and were recorded in metres (*m*). PTT was determined as the foot of the pressure wave corresponding to the foot of the systolic upstroke of the CCA and femoral arterial signals relative to the R-wave of a gated ECG and was recorded in seconds (*s*). Arterial signals were passed through a band-pass filter of 5-30 Hz and the foot of the waveform was

identified as the minimum value of the filtered signal. Three consecutive sequences of at least 15 PP waveforms were obtained at both the CCA and femoral arterial sites using the handheld tonometer, and 15 of the most well-defined and consistent waveforms were averaged in the calculation of the PTT. Values for cfPWV are expressed in m/s.

3.2 Assessment of Serum C-Reactive Protein Concentration

Serum concentrations of CRP were measured and utilized as a marker of inflammation in the current analyses, as subclinical inflammation has been associated with arterial stiffness (Schumacher et al., 2009; Yasmin et al., 2004), and both TL (Wong et al., 2014) and mtDNAcn (Knez et al., 2017). Once CV assessment was complete, venous blood was drawn from the antecubital fossa of each participant into an SST^{TM} Serum Separation Tube (BD Biosciences, Mississauga, ON, Canada) by a licensed nurse. Samples were then allowed to clot at room temperature for approximately 30 minutes. Serum was then separated from 5 mL of blood by centrifugation (1500 g at 4^{o}C for 10 minutes) and 200 µL of sample was aliquoted into several (6 or 7) 1.5 mL microcentrifuge tubes. Serum samples were stored at -80^{o}C until further analysis. A commercially available quantitative enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) was used for the quantification of serum CRP concentrations. First, 20 mL of calibrator diluent RD5P was added to 80 mL of distilled water in order to establish a negative control. Following this, 200 µL of calibrator diluent RD5P was transferred into six 1.5 mL microcentrifuge tubes. A serial dilution of CRP standard was then prepared by loading 200 µL of 50 ng/mL CRP standard into the six microcentrifuge tubes according to Figure 14.

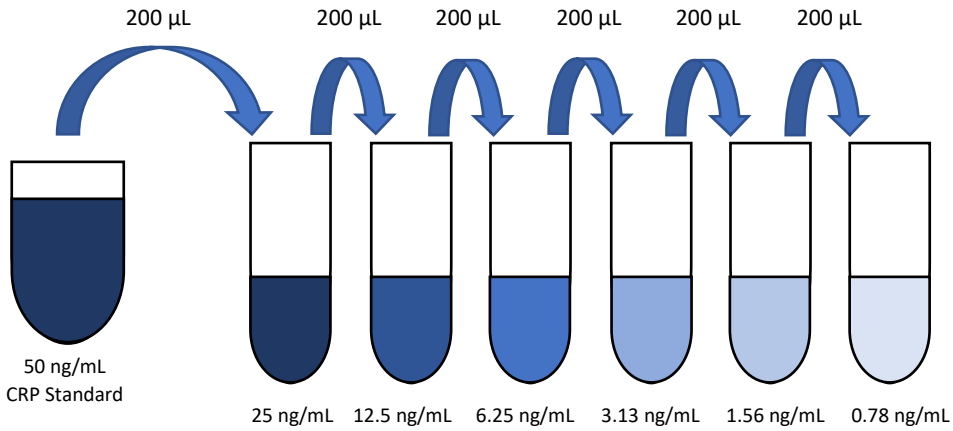

Figure 14. Serial dilution of human CRP standard.

Next, participant serum samples were diluted 100-fold in order to minimize non-specific antibody binding. 100 µL of assay diluent RD1F was added to each well of a 96-well CRP microplate coated with a monoclonal antibody specific to human CRP, and 50 µL of CRP standard, control, and participant sample were added to their respective wells. Following this, the microplate was covered with an adhesive strip and incubated at room temperature for 2 hours. Post-incubation, the adhesive strip was removed and 400 µL of wash buffer was added to each well using a manual plate washer. The liquid was then discarded, and the microplate was blotted using an absorbent towel in order to remove any residual liquid. The washing process was performed for a total of four times. Following this, 200 µL of CRP conjugate (i.e., monoclonal antibody specific for human CRP conjugated to horseradish peroxidase) was added to each well, and the microplate was once again covered with an adhesive strip and incubated at room temperature for 2 hours. The washing process was then repeated four more times. Next, a substrate solution was prepared by mixing 10 mL of colour reagent A (i.e., hydrogen peroxide) with 10 mL of colour reagent B (i.e., chromogen). 200 µL of substrate solution was subsequently added to each well, and the microplate was then covered and incubated at room temperature for an additional 30 minutes. During this incubation period, substrate will have bound to CRP conjugate, which causes a colour change (i.e., colourless to blue) based on the amount of substrate-CRP conjugate binding and,

theoretically, the initial concentration of serum CRP. Following this, 50 µL of stop solution was added to each well in order to terminate the substrate-CRP conjugate reaction, resulting in another colour change from blue to yellow. A microplate reader was then used to determine the optical density of each sample at 450 nm wavelength light with a 540 nm wavelength correction. Optical densities were then compared to the optical densities of a CRP standard in order to determine participant serum CRP concentrations. Greater optical densities were indicative of higher serum CRP concentrations.

3.3 Absolute Telomere Length and Mitochondrial DNA Copy Number Quantification

3.3.1 Saliva Collection

Once CV assessment was complete, participants were instructed to spit into the funnel of a saliva collector tube until the amount of liquid saliva (not bubbles) reached the fill line (i.e., 2 mL of liquid sample) (Oragene·DNA, DNA Genotek, Ottawa, ON, Canada). Participants were required to refrain from eating, drinking, smoking, or chewing gum for at least 30 minutes prior to giving a saliva sample; however, this was not an issue as the participant was undergoing CV assessment the hour beforehand. Once the fill line was reached, the funnel lid was closed, and a stabilizing liquid was discharged into the sample in order to disrupt the integrity of cells within the sample and release their DNA into a controlled liquid environment. The liquid-stabilized environment was optimal for controlling any pre-analytical changes in the sample due to fluctuating temperatures and/or storage durations and conditions. Once the liquid was added, the funnel was removed and replaced with a normal cap. The capped sample was then mixed by shaking the tube for approximately 5 seconds and stored at room temperature.

3.3.2 DNA Isolation

Prior to DNA isolation, saliva samples were incubated for 2 hours at 50^{o}C. Following incubation, 200 µL of each saliva sample was transferred into a 1.5 mL microcentrifuge tube. DNA isolation was then carried out using DNeasy Blood & Tissue Kits (QIAGEN Inc., Toronto, ON, Canada). First, 20 µL of

Proteinase K was added to each saliva sample. Following this, 200 µL of Buffer AL and 200 µL of 100% ethanol were added to each sample, which were then mixed thoroughly. The mixture was then transferred to a spin column placed in a 2 mL collection tube and centrifuged at 8000 rpm for 1 minute. The flow-through and collection tube were discarded, and the spin column was placed in a new 2 mL collection tube. Following this, 500 µL of Buffer AW1 was added to the spin column, and the tube was once again centrifuged at 8000 rpm for 1 minute. The flow-through and collection tube were again discarded, and the spin column was transferred to a new 2 mL collection tube in which 500 µL of Buffer AW2 was added. The tube was then centrifuged at 13,500 rpm for 3 minutes and the flow-through and collection tube were discarded. The spin column was then transferred to a 1.5 mL microcentrifuge tube and 200 µL of Buffer AE was added to the column membrane in order to elute the DNA. The tube was centrifuged at 8000 rpm for 1 minute and the isolated DNA in eluate was stored at -20^{o}C until further analysis.

3.3.3 Determination of DNA Purity

DNA purity was assessed by determining the absorbance ratio of the DNA in eluate at 260 and 280 nm light using spectrophotometry (NanoVue Plus Spectrophotometer, GE Healthcare Life Sciences, Mississauga, ON, Canada). A ratio of 1.8 to 2.0 is generally accepted as pure DNA (Lucena-Aguilar et al., 2016; Olson & Morrow, 2012). If the ratio is appreciably low (≤ 1.6), it may indicate the presence of proteins or other contaminants that absorb strongly at or near 280 nm (Lucena-Aguilar et al., 2016; Olson & Morrow, 2012). Alternatively, if the ratio is appreciably high (≥ 2.0), it may indicate the presence of RNA (Lucena-Aguilar et al., 2016; Olson & Morrow, 2012). If the absorbance ratio fell outside of the accepted ratio for pure DNA, DNA isolation was repeated, and purity was re-assessed.

3.3.4 Absolute Telomere Length and Mitochondrial DNA Copy Number Quantification

Absolute TL and mtDNAcn were quantified using a qPCR assay (Absolute Human Telomere Length and Mitochondrial DNA Copy Number Dual Quantification qPCR Assay Kit, ScienCell Research

Laboratories, Carlsbad, CA, USA). This assay was designed to simultaneously quantify average TL and mtDNAcn of a human cell population using qPCR. A reference genomic DNA sample with known TL and mtDNAcn served as a reference for calculating absolute TL and mtDNAcn of target samples.

3.3.5 Primers

Due to company patent restrictions, the exact sequences of the primers that were used could not be disclosed to the researchers. However, the telomere primer set was designed to recognize and amplify the human telomeric sequence [i.e., 5'-$(TTAGGG)_n$-3'], and was likely derived from the original telomere primer set developed by Richard Cawthon (Cawthon, 2002). These primer sequences were as follows: forward primer, 5'-GGTTTTTGAGGGTGAGGGTGAGGGGAGGGTGAGGGT-3'; reverse primer, 5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3' (Cawthon, 2002). The primers were designed to specifically amplify the telomeric sequence without generating primer dimer-derived products (Cawthon, 2002). The mtDNA primer set was designed to recognize and amplify one of the most conserved regions on human mtDNA. Prior studies have utilized different regions of human mtDNA to quantify mtDNAcn, including a 107-bp mitochondrial fragment of a tRNA gene and a 221-bp mitochondrial fragment at an undisclosed location (Gonzalez-Hunt et al., 2016). Importantly, all mitochondrial primers are designed to amplify only the conserved region of mtDNA and will not amplify any off-target sequences (Gonzalez-Hunt et al., 2016). The single copy reference (SCR) primer set was designed to recognize and amplify a 100-bp sequence on human chromosome 17, and was used for data normalization (Cawthon, 2002). A commonly used SCR gene is the *36B4* gene on chromosome 12 (Cawthon, 2002; O'Callaghan & Fenech, 2011). Primer sets used in this study were validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity, and by template serial dilution for amplification efficiency.

3.3.6 qPCR Procedure

First, each primer set was centrifuged at 3600 rpm for 1 minute. Following centrifugation, 200 µL of nuclease-free H_2O was added to each primer in order to make the primer stock solution. Primer stock solution that was not being used was stored at -20°C. For the reference and participant genomic DNA samples, three qPCR reactions, one with telomere primer stock solution, one with mtDNA primer stock solution, and one with SCR primer stock solution, were prepared. A total of 50 µL of qPCR reaction mixture was prepared for each amplicon (i.e., telomeric sequence, mtDNA sequence, and SCR sequence) in accordance with Table 2 and negative controls (i.e., blanks) were prepared in accordance with Table 3. KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems Inc., Wilmington, MA, USA) was used in each qPCR reaction.

Table 2. Volume composition of qPCR reaction mixture.

Reagent:	Volume (µL):
Reference/Participant Genomic DNA Sample	2.5
Primer Stock Solution	5.0
KAPA SYBR Fast qPCR Master Mix	25.0
Nuclease-Free H_2O	17.5
Total Volume:	50.0

Table 3. Volume composition of negative controls.

Reagent:	Volume (µL):
Primer Stock Solution	3.5
KAPA SYBR Fast qPCR Master Mix	17.5
Nuclease-Free H_2O	14.0
Total Volume:	35.0

A total of 15 µL of each qPCR reaction mixture (i.e., telomeric, mitochondrial, and SCR for the reference and participant genomic DNA samples) was added to the wells of a 96-well qPCR plate in accordance with Figure 15. Reference and participant reactions were run in triplicate, and negative controls were run in duplicate, and the average of there values were used in the calculation of absolute

TL and mtDNAcn. Each plate was able to run reactions for nine participants along with the reference genomic DNA and negative controls.

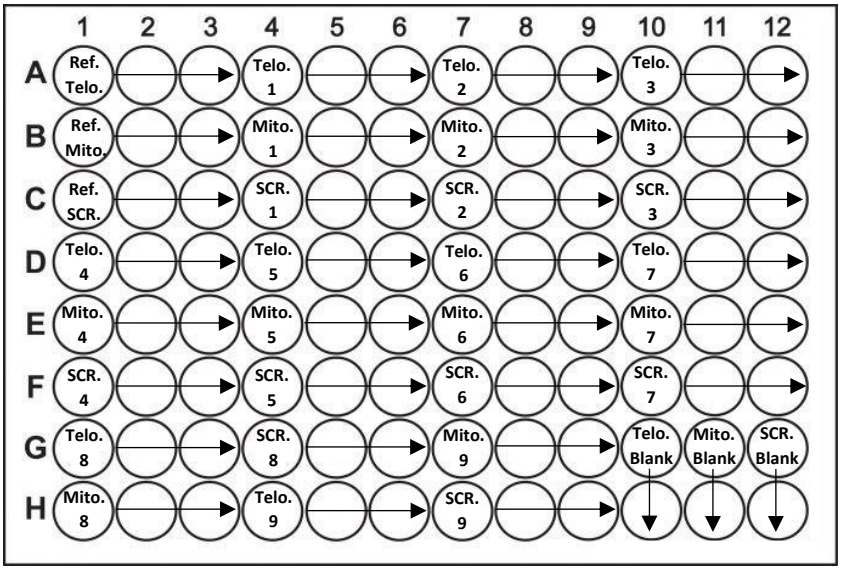

Figure 15. Layout of 96-well plate for qPCR analysis.

Once the qPCR reaction wells were set up, the 96-well plate was sealed and centrifuged at 3600 rpm for approximately 20 seconds to bring all liquid to the bottom of the wells. The qPCR reaction plate was then placed in a thermocycler (StepOnePlus Real-Time PCR System, Applied Biosystems, ThermoFisher Scientific, Mississauga, ON, Canada) equipped with software used to run the plate in accordance with Table 4. An outline of the qPCR thermocycling profile is shown in Figure 16.

Table 4. qPCR thermocycling profile

Step:	Temperature (°C):	Time (s):
Denaturation	95	20
Annealing	60	20
Extension	72	45

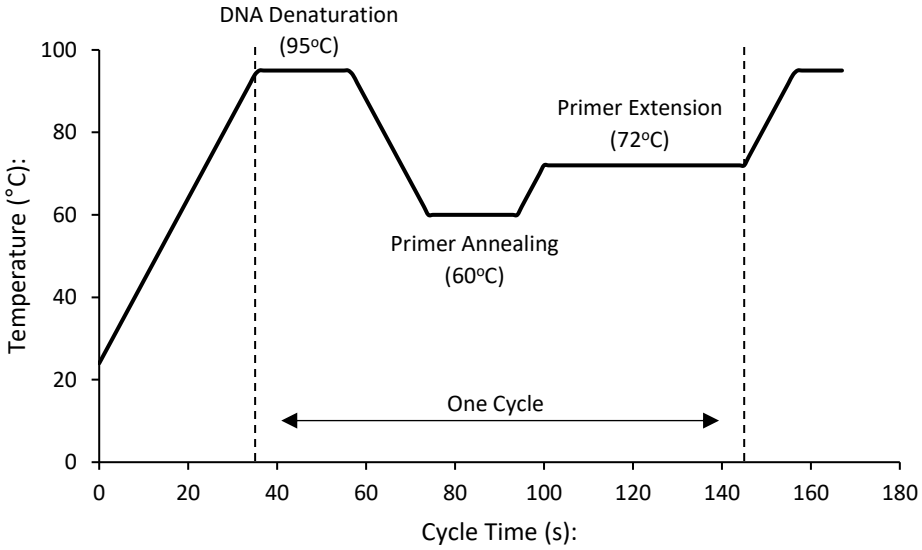

Figure 16. Thermocycling profile of the qPCR procedure.

The thermocycling protocol was carried out for 32 cycles, during which time the fluorescence intensity and threshold cycle (Ct) were recorded. Following qPCR, a melt curve analysis was performed in order to examine primer specificity. Using the Ct values generated for the target and reference genomic samples, a comparative threshold cycle value (ΔΔCt) analysis was performed to determine mean absolute TL and mtDNAcn. A brief overview of the procedure beginning with saliva collection and concluding with the comparative threshold cycle analyses is shown in Figure 17.

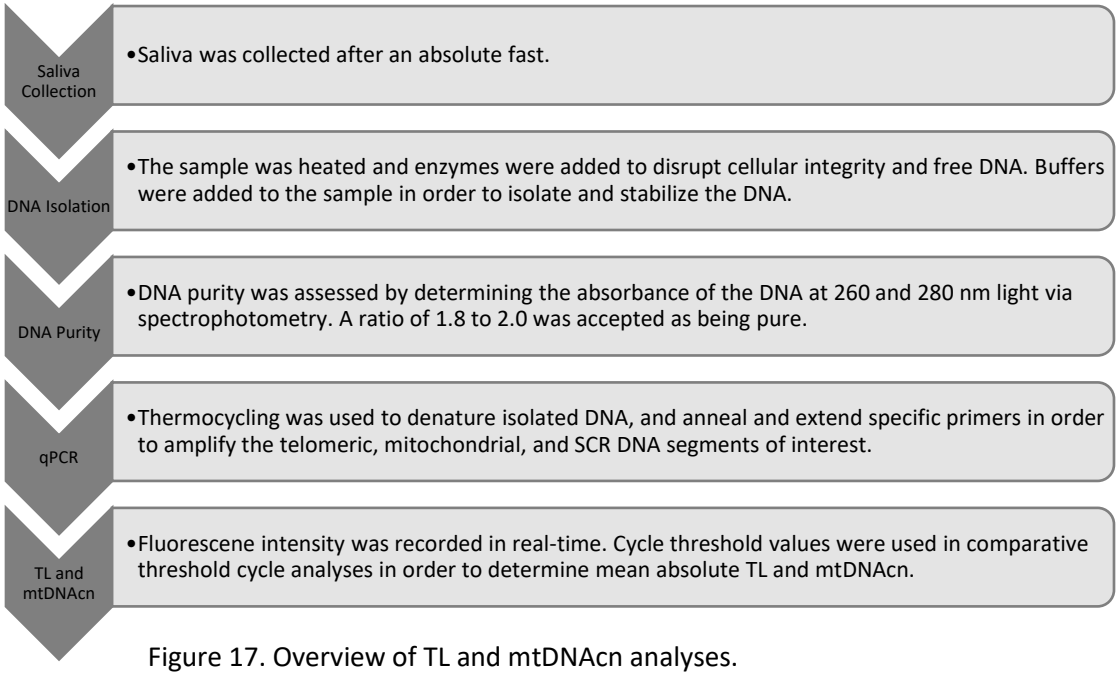

Figure 17. Overview of TL and mtDNAcn analyses.

3.4 Adverse Childhood Experiences

The Childhood Trust Event Survey version 2.0 (CTES 2.0) was used to assess ACEs among participants. Modelled after the original Kaiser ACEs inventory (Felitti et al., 1998), this survey is a 26-item questionnaire that provides information on childhood maltreatment and household dysfunction, as well as other stressful childhood experiences, such as bullying, neighbourhood violence, separation from parents and family, severe material disadvantage, homelessness, natural disasters, and serious illnesses, accidents, and injuries (Edwards et al., 2007; Wade et al., 2019). The CTES 2.0 was self-reported in order to minimize participant discomfort and social response bias due to the sensitivity of the questions (Edwards et al., 2001, 2007). Moreover, the questionnaire was administered at the end of the laboratory visit as well as near the end of the broader questionnaire package. At this time, the researchers had already built a rapport with the participant and, ideally, created an optimal environment for the administration of the CTES 2.0. It has been reported that when conducted in a dignified manner, there is a high level of compliance, reliability, and accuracy in reporting childhood maltreatment, household

dysfunction, and other ACEs (Dube et al., 2004; Edwards et al., 2007). Thus, building a welcoming and trusting environment for the participant should mitigate any unwillingness to report ACEs.

Finally, the CTES 2.0 was part of a much broader questionnaire package administered to the participant. This questionnaire package was multifaceted and included questions pertaining to participant demographics, behaviours, family history of disease, diet and exercise habits, and mental health status, among other themes. Other factors that may influence central artery stiffness or biological markers of stress, such as smoking status and PA level, were also obtained through the broader questionnaire package. Information on smoking status was collected by asking the question: "*Do you currently smoke cigarettes daily, occasionally or not at all?"* Smoking status was then categorized into three groups: regular smokers, occasional smokers, and non-smokers. Regular smokers were defined as those who currently smoked at least one cigarette per day, occasional smokers were defined as those who had smoked less than one cigarette per day, and non-smokers were defined as those who did not currently smoke cigarettes. PA levels were assessed using the International Physical Activity Questionnaire short form (IPAQ-SF), which is a 7-item questionnaire that aims to assess an individuals' PA level over the past 7 days in metabolic equivalents (METs). The IPAQ-SF has been shown to have good validity and reliability (Craig et al., 2003; Lee et al., 2011). PA level was categorized as low active (< 600 MET-minutes/week), moderate active (600-2999 MET-minutes/week), and high active (≥ 3000 MET-minutes/week). For a detailed overview of the broader questionnaire package, see Wade et al. (2019).

3.5 Statistical Analyses

Continuous variables are presented as the mean ± standard deviation (SD) and categorical variables are presented as proportions and relative frequencies (%). Shapiro-Wilk tests of normality were used to assess the distribution of values for continuous variables. Data pertaining to the distribution of TL, mtDNAcn, and CRP were significantly skewed. As such, these variables were log-

transformed in order to better approximate a normal distribution. The log-transformed values were used in subsequent analyses. Pearson correlation coefficients were calculated to assess bivariate associations between continuous study variables. Student's *T*-tests were used to examine differences in mean cfPWV, mean TL, and mean mtDNAcn between sexes, and one-way analyses of variance (ANOVA) followed by Tukey's *post-hoc* tests were used to examine differences in mean cfPWV, mean TL, and mean mtDNAcn between smoking status groups, BMI classification, PA groups, and ACE categorization. ACEs were categorized into 0, 1, 2, 3, or ≥ 4 experiences. Multiple linear regression analyses (MLRA) were used to examine the association between ACEs and cfPWV, ACEs and TL, ACEs and mtDNAcn, TL and cfPWV, and mtDNAcn and cfPWV. Age, sex, smoking status, BMI, PA, instantaneous MAP and HR, and serum CRP concentration were identified *a priori* as potential covariates in the association between ACEs, cfPWV, and markers of biological stress, and as such, they were adjusted for in MLRA. The interaction terms ACEs by sex, smoking status, BMI classification, and PA level, TL by sex, smoking status, BMI classification, and PA level, and mtDNAcn by sex, smoking status, BMI classification, and PA level were analyzed in subsequent regression models in order to determine the potential moderator effects between these variables in predicting cfPWV. Sex, smoking status, BMI, and PA were selected as potential moderators due to their associations with the main study variables (i.e., ACEs, TL, mtDNAcn, and cfPWV), which were identified in the literature review. Additionally, these variables were selected based on their classification as being lifestyle factors (except for sex). Finally, the potential mediating effects of TL and mtDNAcn on the association between ACEs and cfPWV were explored using the mediation analysis proposed by Baron and Kenny (1986) (Baron & Kenny, 1986). The assumption of normality of residuals was examined using Shapiro-Wilk tests of normality, the assumption of homogeneity of variance was examined by plotting residuals versus predicted values and via White tests, the assumption of collinearity was examined by assessing variance inflation factors and tolerance values, and the assumption of independence of residuals was examined by assessing the Durbin-Watson

statistic. All assumptions of ANOVA and MLRA were met. The sample size required to detect a small-medium effect size (Cohen's $f^2 = 0.10$) at an α-level of 0.05 and a power ($1 - \beta$) of 0.80 was 172 individuals (Faul et al., 2009). *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

Chapter IV: Results

4.1 Participant Characteristics

The characteristics of the study population can be observed in Table 5. A total of 185 participants (*n* = 102 females) were included in the current analyses. Mean cfPWV of the study population was 5.8 ± 0.9 m/s, which is considered normal with respect to The Reference Values for Arterial Stiffness' Collaboration (2010) (The Reference Values for Arterial Stiffness' Collaboration, 2010). Mean TL and mean mtDNAcn of the study population were 649.9 ± 799.4 kb per diploid cell and 624.0 ± 469.2 copies per diploid cell, respectively. Mean log(TL) and mean log(mtDNAcn) were 2.6 ± 0.4 kb per diploid cell and 2.7 ± 0.3 copies per diploid cell, respectively. The mean number of ACEs in the current study population was 2.3 ± 2.1 ACEs. Within this population, 32 individuals (17.3%) had reported experiencing zero ACEs, 51 individuals (27.6%) had reported experiencing one ACE, 33 individuals (17.8%) had reported experiencing two ACEs, 23 individuals (12.4%) had reported experiencing three ACEs, and 46 individuals (24.9%) had reported experiencing four or more ACEs. The distribution of ACEs by type can be observed in Table 6.

Table 5. Characteristics of the study population (*n* = 185).

Variable	Parameter	Range
Age (Years)	22.5 ± 1.5	19.4 – 25.5
Sex [n(%)]		
Males	83 (44.9)	-
Females	102 (55.1)	-
Smoking Status [n(%)]		
Daily Smokers	13 (7.0)	-
Occasional Smokers	12 (6.5)	-
Non-Smokers	160 (86.5)	-
BMI (kg/m^2)	25.2 ± 5.4	16.4 – 43.9
BMI Classification [n(%)]		
Normal Weight	106 (57.3)	-
Overweight	51 (27.6)	-
Obese	28 (15.1)	-
PA (MET-minutes/week)	8798.6 ± 8833.5	165.0 – 53,004.0
MAP (mmHg)	81.6 ± 8.7	61.2 – 103.5
SBP (mmHg)	114.1 ± 10.7	86.1 – 146.4
DBP (mmHg)	65.3 ± 9.4	41.2 – 88.8
HR (bpm)	65.4 ± 9.9	38.9 – 94.4
cfPWV (m/s)	5.8 ± 0.9	3.7 – 8.3
CRP (ng/mL)	3.0 ± 4.6	0.1 – 25.0
Log(CRP)	0.1 ± 0.6	0.1 – 1.4
TL (kb per diploid cell)	649.9 ± 799.4	67.5 – 3,745.7
Log(TL)	2.6 ± 0.4	1.8 – 3.6
mtDNAcn (copy number per diploid cell)	624.0 ± 469.2	102.7 – 2883.3
Log(mtDNAcn)	2.7 ± 0.3	2.0 – 3.5
ACEs (number of experiences)	2.3 ± 2.1	0 – 8.0
ACEs [n(%)]		
0	32 (17.3)	-
1	51 (27.6)	-
2	33 (17.8)	-
3	23 (12.4)	-
≥4	46 (24.9)	-

*Continuous variables are presented as mean values ± SD, and categorical variables are presented as proportions and relative frequencies.

†BMI indicates body mass index; PA, physical activity; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; cfPWV, carotid-femoral pulse wave velocity; CRP; serum C-reactive protein concentration; Log(CRP), log-transformed CRP; TL, absolute telomere length; log(TL), log-transformed absolute telomere length; mtDNAcn, mitochondrial DNA copy number; log(mtDNAcn), log-transformed absolute mitochondrial DNA copy number; ACEs, adverse childhood experiences.

Table 6. Distribution of ACEs by type (*n* = 185).

ACE Type	Parameter [n(%)]
Any Form of Abuse	
Yes	85 (46.0)
No	100 (54.0)
Physical Abuse	
Yes	35 (18.9)
No	150 (81.1)
Sexual Abuse	
Yes	36 (19.5)
No	149 (80.5)
Emotional Abuse	
Yes	68 (36.8)
No	117 (63.2)
Any Form of Household Dysfunction	
Yes	133 (71.9)
No	52 (28.1)
Loss of Someone Close/Separated from a Parent(s)	
Yes	86 (46.5)
No	99 (53.5)
Domestic Violence within the Home	
Yes	42 (22.7)
No	143 (77.3)
Drug/Alcohol Misuse within the Home	
Yes	50 (27.0)
No	135 (73.0)
Family Member Imprisoned	
Yes	29 (15.7)
No	156 (84.3)
Mental Illness within the Home	
Yes	87 (47.0)
No	98 (53.0)

*Variables are presented as proportions and relative frequencies [n(%)].

4.2 Bivariate and Between-Groups Analyses

In bivariate analysis, cfPWV was significantly associated with ACEs ($r = 0.201$, $p = 0.006$) (Table 7). Additionally, cfPWV was positively associated with age ($r = 0.205$, $p = 0.005$), BMI ($r = 0.306$, $p < 0.001$), MAP ($r = 0.232$, $p = 0.002$), SBP ($r = 0.273$, $p < 0.001$), DBP ($r = 0.164$, $p = 0.025$), and HR ($r = 0.202$, $p = 0.006$). With respect to biological measures, cfPWV was positively associated with serum CRP concentration ($r = 0.178$, $p = 0.015$), and negatively associated with TL ($r = -0.176$, $p = 0.016$) (Figure 18). cfPWV was not associated with mtDNAcn; however, the relationship appeared to be trending in the negative direction ($r = -0.142$, $p = 0.054$) (Figure 19).

Between-groups analyses revealed that mean cfPWV did not significantly differ by sex nor PA level (both $p > 0.05$). Mean cfPWV significantly differed by smoking status, such that regular smokers had a significantly faster mean cfPWV compared to non-smokers (6.35 ± 0.84 *vs.* 5.78 ± 0.86 m/s, respectively; $p = 0.026$), but not occasional smokers (6.35 ± 0.84 *vs.* 6.15 ± 1.14 m/s, respectively; $p > 0.05$). There was no significant difference in mean cfPWV between occasional smokers and non-smokers ($p > 0.05$). In addition to smoking status, mean cfPWV significantly differed by BMI classification, such that those classified as obese (BMI ≥ 30 kg/m^2) had a significantly faster mean cfPWV compared to those classified as normal weight (BMI < 25 kg/m^2) (6.32 ± 1.13 *vs.* 5.70 ± 0.84 m/s, respectively; $p = 0.003$). There was no significant difference in mean cfPWV between those classified as obese and those classified as overweight (25 kg/m^2 ≤ BMI < 30 kg m^2) (6.32 ± 1.13 *vs.* 5.88 ± 0.75 m/s, respectively; $p > 0.05$); nor was there a difference in mean cfPWV between those classified as overweight and those classified as normal weight ($p > 0.05$). Finally, mean cfPWV significantly differed by ACE groupings, such that those who had reported experiencing four or more ACEs had a significantly faster mean cfPWV compared to those who had not reported experiencing any ACEs (6.17 ± 0.91 *vs.* 5.58 ± 0.92 m/s, respectively; $p = 0.030$) (Figure 20). There was no significant difference in mean cfPWV between any other ACE grouping (all $p > 0.05$).

TL was significantly positively associated with mtDNAcn (r = 0.558, p < 0.001) (Table 7; Figure 21). TL was not associated with any other study variable in the current analysis (all p > 0.05). In contrast, mtDNAcn was significantly positively associated with PA (r = 0.150, p = 0.041), but no other study variables (all p > 0.05) (Table 7). Between-groups analyses revealed that mean TL did not significantly differ by sex, smoking status, BMI classification, nor PA level (all p > 0.05). Additionally, mean TL did not significantly differ by ACE grouping (p > 0.05) (Figure 22).

Similar to TL, mean mtDNAcn did not significantly differ by sex, BMI classification, nor PA level (all p > 0.05); however, mean mtDNAcn significantly differed by smoking status, such that regular smokers had a significantly lower mean mtDNAcn compared to non-smokers (597.13 ± 36.40 *vs.* 995.14 ± 127.70 copies per diploid cell, respectively; p = 0.009), but not occasional smokers (597. 13 ± 36.40 *vs.* 579.94 ± 132.91 copies per diploid cell, respectively; p > 0.05). There was no significant difference in mean mtDNAcn between occasional and non-smokers (p > 0.05). Mean mtDNAcn did not significantly differ by ACE grouping (p > 0.05) (Figure 23).

In bivariate analysis, ACEs were significantly positively associated with serum CRP concentration (r = 0.153, p = 0.037) (Table 7). ACEs were not significantly associated with any other study variable in the current analysis (all p > 0.05); however, the association between ACEs and PA appeared to be trending in the positive direction (r = 0.138, p = 0.061). Between-groups analyses revealed that mean number of ACEs did not differ by sex, BMI classification, nor PA level (all p > 0.05). However, mean number of ACEs significantly differed by smoking status, such that regular smokers had reported experiencing a greater mean number of ACEs compared to non-smokers (3.23 ± 1.24 *vs*. 1.89 ± 1.41 ACEs, respectively; p = 0.003), but not occasional smokers (3.23 ± 1.24 *vs*. 2.17 ± 1.59 ACEs, respectively; p > 0.05). There was no significant difference in mean number of ACEs between occasional and non-smokers (p > 0.05).

Table 7. Bivariate associations between study variables (*n* = 185).

	Pearson Correlation Coefficients (*r*) *p*-value										
	Age (Years)	PA	BMI	MAP	SBP	DBP	HR	cfPWV	CRP	TL	mtDNAcn	ACEs
Age (Years)	-	0.108 *0.142*	-0.007 *0.929*	-0.025 *0.734*	0.089 *0.229*	-0.085 *0.250*	-0.028 *0.707*	0.205 ***0.005***	-0.064 *0.389*	-0.026 *0.726*	-0.030 *0.686*	0.079 *0.285*
PA		-	-0.035 *0.640*	-0.018 *0.806*	0.045 *0.547*	-0.050 *0.497*	-0.044 *0.554*	-0.062 *0.400*	-0.146 ***0.047***	0.064 *0.386*	0.150 ***0.041***	0.138 *0.061*
BMI			-	0.347 ***<0.001***	0.335 ***<0.001***	0.288 ***<0.001***	0.152 ***0.039***	0.306 ***<0.001***	0.437 ***<0.001***	0.016 *0.827*	-0.008 *0.918*	0.088 *0.236*
MAP				-	0.779 ***<0.001***	0.934 ***<0.001***	0.172 ***0.019***	0.232 ***0.002***	0.047 *0.527*	0.006 *0.932*	0.030 *0.682*	-0.059 *0.428*
SBP					-	0.505 ***<0.001***	0.053 *0.478*	0.273 ***<0.001***	0.002 *0.981*	0.023 *0.756*	-0.005 *0.944*	-0.052 *0.484*
DBP						-	0.207 ***0.005***	0.164 ***0.025***	0.063 *0.391*	-0.004 *0.953*	0.045 *0.546*	-0.051 *0.488*
HR							-	0.202 ***0.006***	0.286 ***<0.001***	-0.066 *0.372*	-0.021 *0.777*	0.083 *0.263*
cfPWV								-	0.178 ***0.015***	-0.176 ***0.016***	-0.142 *0.054*	0.201 ***0.006***
CRP									-	-0.020 *0.791*	-0.003 *0.973*	0.153 ***0.037***
TL										-	0.558 ***<0.001***	-0.014 *0.846*
mtDNAcn											-	0.017 *0.820*
ACEs												-

*$p < 0.05$ indicates significance.

†PA indicates physical activity (MET-minutes/week); BMI, body mass index (kg/m^2); MAP, mean arterial pressure (mmHg); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); HR, heart rate (bpm); cfPWV, carotid-femoral pulse wave velocity (m/s); CRP, log-transformed C-reactive protein (ng/mL); TL, log-transformed absolute telomere length (kb per diploid cell); mtDNAcn, log-transformed mitochondrial DNA copy number (copies per diploid cell); ACEs, adverse childhood experiences (0, 1, 2, 3, or ≥ 4).

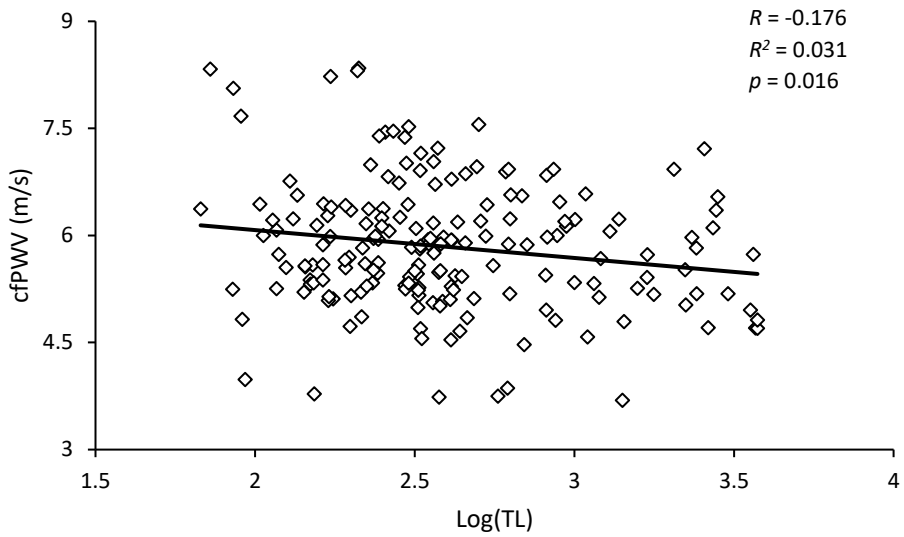

Figure 18. Correlation of carotid-femoral pulse wave velocity [cfPWV (m/s)] with log-transformed telomere length [log(TL)].

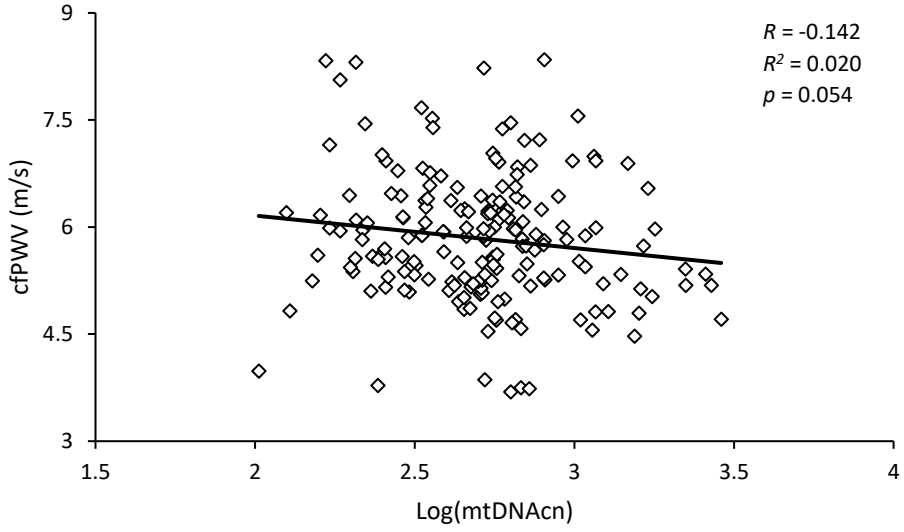

Figure 19. Correlation of carotid-femoral pulse wave velocity [cfPWV (m/s)] with log-transformed mitochondrial DNA copy number [log(mtDNAcn)].

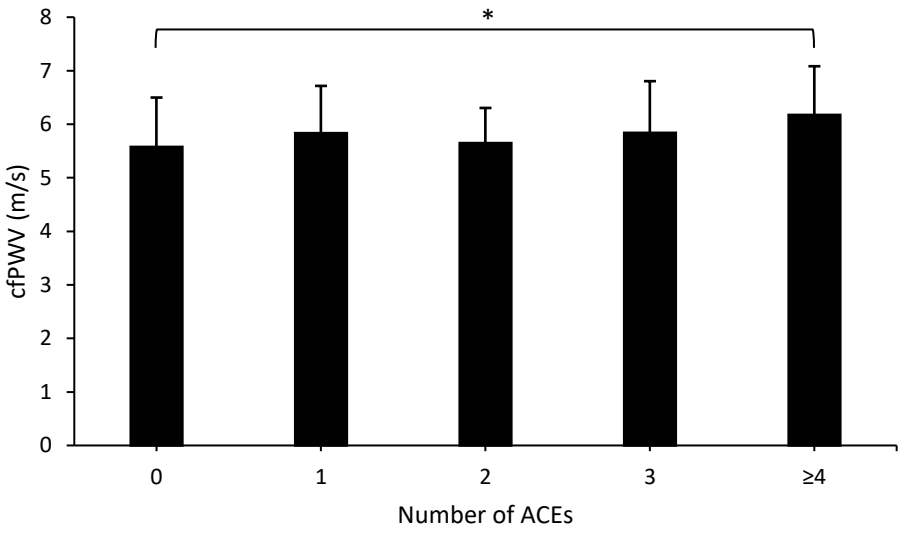

Figure 20. Mean carotid-femoral pulse wave velocity (cfPWV) by number of adverse childhood experiences (ACEs). 0 ACEs, *n* = 32; 1 ACE, *n* = 51; 2 ACEs, *n* = 33; 3 ACEs, *n* = 23; ≥ 4 ACEs, *n* = 46. Error bars represent the SD. "*" indicates significance ($p < 0.05$).

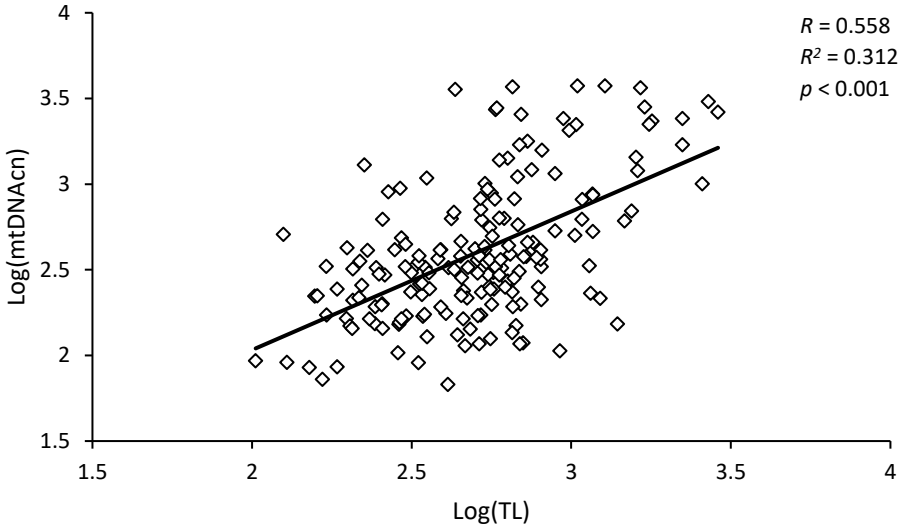

Figure 21. Correlation of log-transformed mitochondrial DNA copy number [log(mtDNAcn) with log-transformed telomere length [log(TL)].

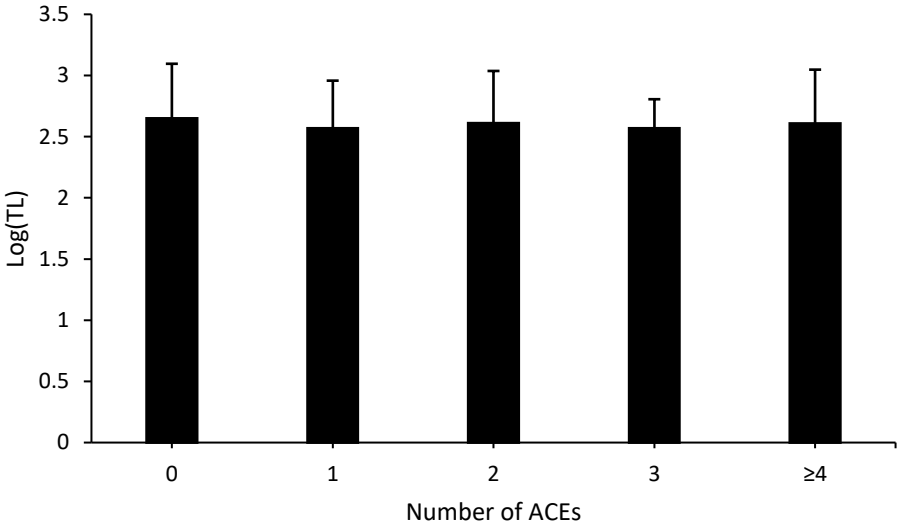

Figure 22. Mean log-transformed telomere length [log(TL)] by number of adverse childhood experiences (ACEs). 0 ACEs, *n* = 32; 1 ACE, *n* = 51; 2 ACEs, *n* = 33; 3 ACEs, *n* = 23; ≥ 4 ACEs, *n* = 46. Error bars represent the SD. "*" indicates significance ($p < 0.05$).

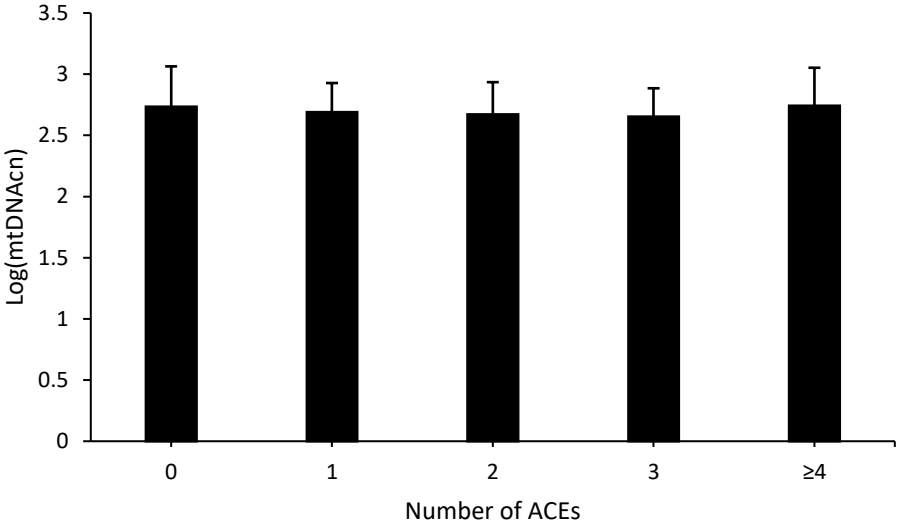

Figure 23. Mean log-transformed mitochondrial DNA copy number [log(mtDNAcn)] by number of adverse childhood experiences (ACEs). 0 ACEs, *n* = 32; 1 ACE, *n* = 51; 2 ACEs, *n* = 33; 3 ACEs, *n* = 23; ≥ 4 ACEs, *n* = 46. Error bars represent the SD . "*" indicates significance ($p < 0.05$).

4.3 Linear Regression Main Effect of ACEs and Covariates on cfPWV

Multiple linear regression analyses were used to examine the association of ACEs and cfPWV after adjustment for several covariates (i.e., age, sex, smoking status, BMI, PA, instantaneous MAP and HR, and serum CRP) (Table 8). ACEs were a significant and positive predictor of cfPWV (β = 0.146, p = 0.038), independent of age, sex, smoking status, BMI, PA, MAP, HR, and serum CRP (Table 8). Age, BMI, PA, and HR were also significant predictors of cfPWV in this model (all $p < 0.05$). Together, age, BMI, PA, HR, and ACEs were able to predict 19.3% of the variation in cfPWV (R^2 = 0.193, $p < 0.001$). Sex, smoking status, MAP, and serum CRP were not predictors of cfPWV in this model (all $p > 0.05$).

Table 8. Linear regression main effect of ACEs and covariates on cfPWV (n = 185).

Model[§] Variables	*B*(*SE*)	*β*	95% CI		*p*
			LL	UL	
Age (Years)	0.111 (0.042)	0.182	0.029	0.194	***0.008***
Sex[†]	0.099 (0.136)	0.056	-0.170	0.369	*0.468*
Occ. Smoke[‡]	0.445 (0.248)	0.124	-0.044	0.934	*0.074*
Reg. Smoke[‡]	0.459 (0.259)	0.132	-0.052	0.970	*0.078*
BMI	0.035 (0.013)	0.215	0.010	0.061	***0.007***
PA	-0.122 (0.061)	-0.143	-0.241	-0.002	***0.047***
MAP	0.013 (0.008)	0.125	-0.003	0.028	*0.102*
HR	0.015 (0.006)	0.170	0.003	0.028	***0.019***
CRP	0.020 (0.130)	0.013	-0.237	0.277	*0.879*
ACEs	0.090 (0.043)	0.146	0.005	0.174	***0.038***

*Reg. Smoke indicates regular smoking; Occ. Smoke, occasional smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum C-reactive protein; ACEs, adverse childhood experiences (0, 1, 2, 3, or ≥ 4). *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model = 0.243, $p < 0.001$.

4.3.1 Interaction Effects of ACEs by Sex, Smoking Status, BMI, and PA on cfPWV

Interaction effects between ACEs and sex (Table 9, Model 1), smoking status (Model 2), BMI (Model 3), and PA (Model 4) on cfPWV were examined in subsequent regression models. There was no significant interaction between either ACEs and sex, nor ACEs and PA in predicting cfPWV (both $p > 0.05$) (Table 9). Model 2 revealed a significant interaction between ACEs and regular smoking ($\beta = 0.421$, $p = 0.032$), but not occasional smoking ($\beta = -0.033$, $p = 0.778$), in predicting cfPWV (Table 9). The ACEs by smoking interaction can be observed in Figure 24. Additionally, Model 3 revealed significant interaction between ACEs and BMI ($\beta = 0.972$, $p = 0.006$) in predicting cfPWV (Table 9). The ACEs by BMI interaction can be observed in Figure 25.

Table 9. Interaction effects of ACEs by sex (Model 1), ACEs by smoking status (Model 2), ACEs by BMI (Model 3), and ACEs by PA (Model 4) on cfPWV (*n* = 185).

Model	Variable	*B*(*SE*)	*β*	95% CI		*p*
				LL	UL	
Model 1[§]	Age (Years)	0.112 (0.042)	0.183	0.029	0.195	***0.008***
	Sex[†]	0.139 (0.211)	0.078	-0.278	0.556	*0.510*
	Occ. Smoke[‡]	0.449 (0.249)	0.125	-0.042	0.940	*0.073*
	Reg. Smoke[‡]	0.468 (0.262)	0.135	-0.050	0.986	*0.076*
	BMI	0.035 (0.013)	0.214	0.009	0.061	***0.008***
	PA	-0.122 (0.061)	-0.144	-0.242	0.002	*0.054*
	MAP	0.013 (0.008)	0.125	-0.003	0.028	*0.104*
	HR	0.015 (0.006)	0.169	0.002	0.028	***0.020***
	CRP	0.020 (0.131)	0.013	-0.238	0.278	*0.879*
	ACEs	0.099 (0.056)	0.161	-0.013	0.210	*0.082*
	ACEs*Sex	-0.021 (0.083)	-0.032	-0.185	0.143	*0.803*
Model 2[§]	Age (Years)	0.097 (0.042)	0.159	0.014	0.180	***0.022***
	Sex[†]	0.099 (0.135)	0.055	-0.169	0.366	*0.467*
	Occ. Smoke[‡]	0.543 (0.415)	0.151	-0.276	1.362	*0.192*
	Reg. Smoke[‡]	-0.867 (0.662)	-0.250	-2.174	0.440	*0.192*
	BMI	0.041 (0.013)	0.245	0.014	0.067	***0.003***
	PA	-0.113 (0.060)	-0.133	-0.232	0.006	*0.062*
	MAP	0.013 (0.008)	0.123	-0.003	0.028	*0.106*
	HR	0.016 (0.006)	0.175	0.003	0.029	***0.015***
	CRP	0.010 (0.046)	0.006	-0.246	0.266	*0.941*
	ACEs	0.070 (0.046)	0.114	-0.020	0.160	*0.128*
	ACEs*Occ. Smoke	-0.045 (0.159)	-0.033	-0.358	0.268	*0.778*
	ACEs*Reg. Smoke	0.423 (0.195)	0.421	0.038	0.808	***0.032***

Model 3§	Age (Years)	0.100 (0.041)	0.163	0.018	0.181	***0.017***
	Sex†	0.109 (0.134)	0.061	-0.155	0.373	*0.417*
	Occ. Smoke‡	0.372 (0.245)	0.103	-0.111	0.854	*0.131*
	Reg. Smoke‡	0.548 (0.256)	0.158	0.042	1.053	***0.034***
	BMI	-0.012 (0.021)	-0.072	-0.054	0.030	*0.577*
	PA	-0.108 (0.060)	-0.127	-0.226	0.009	*0.071*
	MAP	0.015 (0.008)	0.148	-0.001	0.030	*0.051*
	HR	0.013 (0.006)	0.141	0.001	0.025	***0.049***
	CRP	0.059 (0.129)	0.036	-0.197	0.311	*0.660*
	ACEs	-0.459 (0.201)	-0.748	-0.856	-0.063	***0.024***
	ACEs*BMI	0.021 (0.008)	0.972	0.006	0.036	***0.006***
Model 4§	Age (Years)	0.114 (0.041)	0.187	0.032	0.196	***0.007***
	Sex†	0.094 (0.135)	0.053	-0.173	0.361	*0.487*
	Occ. Smoke‡	0.446 (0.246)	0.124	-0.040	0.931	*0.072*
	Reg. Smoke‡	0.406 (0.258)	0.117	-0.104	0.915	*0.118*
	BMI	0.036 (0.013)	0.218	0.010	0.062	***0.006***
	PA	-0.272 (0.097)	-0.320	-0.463	0.082	*0.054*
	MAP	0.015 (0.008)	0.142	-0.001	0.030	*0.064*
	HR	0.015 (0.006)	0.170	0.003	0.028	***0.018***
	CRP	0.024 (0.129)	0.015	-0.232	0.279	*0.856*
	ACEs	-0.565 (0.332)	-0.920	-1.221	0.091	*0.091*
	ACEs*PA	0.076 (0.038)	1.116	-0.001	0.151	*0.084*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum C-reactive protein (ng/mL); ACEs, adverse childhood experiences (0, 1, 2, 3, or ≥ 4).; ACEs*Sex, ACEs by sex interaction term; ACEs*Occ. Smoke, ACEs by occasional smoking interaction term; ACEs*Reg. Smoke, ACEs by regular smoking interaction term; ACEs*BMI, ACEs by BMI interaction term; ACEs*PA, ACEs by PA interaction term. *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model 1 = 0.244, $p < 0.001$; R^2 of Model 2 = 0.264, $p < 0.001$; R^2 of Model 3 = 0.276, $p < 0.001$; R^2 of Model 4 = 0.260, $p < 0.001$.

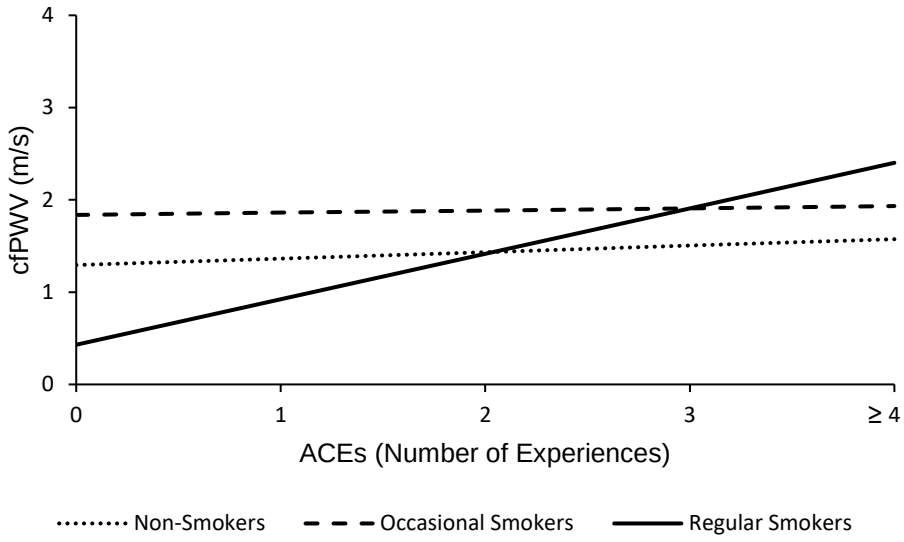

Figure 24. Interaction between adverse childhood experiences (ACEs) and smoking status in predicting carotid-femoral pulse wave velocity (cfPWV).

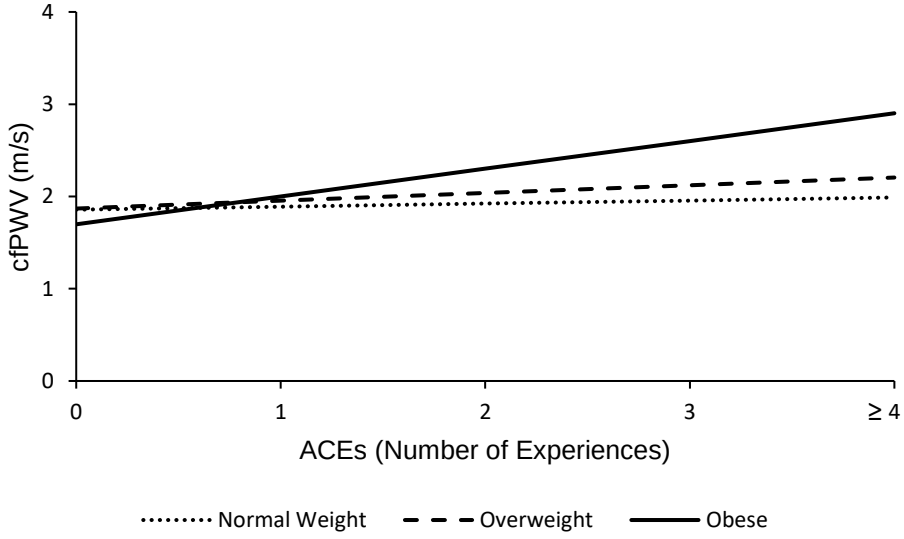

Figure 25. Interaction between adverse childhood experiences (ACEs) and body mass index (BMI) classification in predicting carotid-femoral pulse wave velocity (cfPWV).

4.4 Linear Regression Main Effect of ACEs and Covariates on TL and mtDNAcn

Multiple linear regression analyses were used to examine the association of ACEs on TL and mtDNAcn after adjustment for several covariates (i.e., age, sex, smoking status, BMI, PA, and serum CRP) (Table 10 and Table 11, respectively). ACEs were not a significant predictor of TL (β = -0.027, p = 0.726) (Table 10). Additionally, age, sex, smoking status, BMI, PA, and serum CRP were not significant predictors of TL (all $p > 0.05$). Overall, the model was unable to significantly predict TL (R^2 = 0.028, p = 0.754). Similarly, ACEs were not a significant predictor of mtDNAcn (β = -0.038, p = 0.620) (Table 11). Similarly, age, sex, occasional smoking, BMI, PA, and serum CRP were not significant predictors of mtDNAcn (all $p > 0.05$). Regular smoking was a significant and positive predictor of mtDNAcn in this model (β = 0.210, p = 0.011); however, overall, the model was unable to significantly predict mtDNAcn (R^2 = 0.142, p = 0.066).

Table 10. Linear regression main effect of ACEs and covariates on TL (*n* = 185).

Model§ Variables	*B*(*SE*)	*β*	95% CI		*p*
			LL	UL	
Age (Years)	-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*
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*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); CRP, log-transformed C-reactive protein (ng/mL); ACEs, adverse childhood experiences (0, 1, 2, 3, or ≥ 4). *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of the Model = 0.028, $p = 0.754$.

Table 11. Linear regression main effect of ACEs and covariates on mtDNAcn (*n* = 185).

Model[§] Variables	*B*(*SE*)	*β*	95% CI		*p*
			LL	UL	
Age (Years)	-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*
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*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); CRP, log-transformed C-reactive protein (ng/mL); ACEs, adverse childhood experiences (0, 1, 2, 3, or ≥ 4). *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of the Model = 0.142, $p = 0.066$.

4.5 Linear Regression Main Effect TL, mtDNAcn, and covariates on cfPWV

Multiple linear regression analyses were used to examine the association of TL and mtDNAcn on cfPWV after adjustment for several covariates (i.e., age, sex, smoking status, BMI, PA, instantaneous MAP and HR, and serum CRP (Table 12 and Table 13, respectively). TL was a significant negative predictor of cfPWV (β = -0.169, p = 0.012), independent of age, sex, smoking status, BMI, PA, MAP, HR, and serum CRP (Table 12). Age, regular smoking, BMI, and HR were also significant predictors of cfPWV in this model (all $p > 0.05$). Together, TL, age, regular smoking, BMI, and HR were able to predict 21.0% of the variation in cfPWV (R^2 = 0.210, $p < 0.001$). Sex, occasional smoking, PA, MAP, and serum CRP were not significant predictors of cfPWV in this model (all $p > 0.05$) (Table 12).

In a model that substituted TL for mtDNAcn, mtDNAcn was a significant negative predictor of cfPWV (β = -0.525, p = 0.017), independent of age, sex, smoking status, BMI, PA, MAP, HR, and serum CRP (Table 13). Age, regular smoking, BMI, and HR were also significant predictors of cfPWV in this model (all $p < 0.05$). Together, mtDNAcn, age, regular smoking, BMI, and HR were able to predict 20.5% of the variation in cfPWV (R^2 = 0.205, $p < 0.001$). Sex, occasional smoking, PA, MAP, and serum CRP were not significant predictors of cfPWV in this model (all $p > 0.05$) (Table 13).

Table 12. Linear regression main effect of TL and covariates on cfPWV (n = 185).

Model[§] Variables	B(SE)	β	95% CI		p
			LL	UL	
Age (Years)	0.109 (0.042)	0.177	0.026	0.191	***0.010***
Sex[†]	0.114 (0.136)	0.064	-0.154	0.383	*0.401*
Occ. Smoke[‡]	0.419 (0.247)	0.116	-0.069	0.906	*0.092*
Reg. Smoke[‡]	0.598 (0.253)	0.172	0.098	1.097	***0.019***
BMI	0.038 (0.013)	0.231	0.012	0.064	***0.004***
PA	-0.102 (0.060)	-0.120	-0.220	0.016	*0.091*
MAP	0.012 (0.008)	0.112	-0.004	0.027	*0.139*
HR	0.016 (0.006)	0.172	0.003	0.028	***0.017***
CRP	0.037 (0.129)	0.023	-0.219	0.292	*0.776*
TL	-0.374 (0.148)	-0.169	-0.666	-0.083	***0.012***

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum C-reactive protein (ng/mL); TL, log-transformed absolute telomere length (kb per diploid cell). B indicates the unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; LL, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model = 0.252, $p < 0.001$.

Table 13. Linear regression main effect of mtDNAcn and covariates on cfPWV (*n* = 185).

Model[§] Variables	*B*(*SE*)	*β*	95% CI		*p*
			LL	UL	
Age (Years)	0.105 (0.042)	0.172	0.023	0.188	***0.013***
Sex[†]	0.094 (0.136)	0.052	-0.174	0.362	*0.492*
Occ. Smoke[‡]	0.474 (0.246)	0.132	-0.012	0.960	*0.056*
Reg. Smoke[‡]	0.680 (0.258)	0.196	0.171	1.189	***0.009***
BMI	0.037 (0.013)	0.221	0.012	0.062	***0.006***
PA	-0.093 (0.060)	-0.109	-0.212	0.026	*0.124*
MAP	0.012 (0.008)	0.121	-0.003	0.028	*0.111*
HR	0.016 (0.006)	0.183	0.004	0.029	***0.011***
CRP	0.037 (0.130)	0.023	-0.219	0.293	*0.775*
mtDNAcn	-0.525 (0.218)	-0.164	-0.956	-0.095	***0.017***

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum C-reactive protein (ng/mL); mtDNAcn, log-transformed mitochondrial DNA copy number (copies per diploid cell). *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model = 0.249, $p < 0.001$.

4.5.1 Interaction Effects of TL and mtDNAcn by Sex, Smoking Status, BMI, and PA in Predicting cfPWV

Interactions between TL and sex (Table 14, Model 1), smoking status (Model 2), BMI (Model 3), and PA (Model 4) on cfPWV were examined in subsequent regression models. There was no significant interaction between TL and sex, nor TL and PA in predicting cfPWV (both $p > 0.05$) (Table 14). Model 2 revealed a significant interaction between TL and regular smoking in predicting cfPWV ($\beta = -0.869$, $p = 0.038$), but not occasional smoking ($\beta = -0.907$, $p = 0.053$). The TL by smoking interaction can be observed in Figure 26. Additionally, Model 3 revealed a significant interaction between TL and BMI in predicting cfPWV ($\beta = -1.312$, $p = 0.029$). The TL by BMI interaction can be observed in Figure 27.

Interactions between mtDNAcn and sex (Model 1), smoking status (Model 2), BMI (Model 3), and PA (Model 4) on cfPWV were examined. There was no significant interaction between mtDNAcn and sex, mtDNAcn and BMI, or mtDNAcn and PA in predicting cfPWV (all $p > 0.05$) (Table 15). Model 2 revealed a significant interaction between mtDNAcn and regular smoking in predicting cfPWV ($\beta = -1.758$, $p = 0.022$), but not occasional smoking ($\beta = -0.946$, $p = 0.200$). The mtDNAcn by smoking interaction can be observed in Figure 28.

Table 14. Interaction effects of TL by sex (Model 1), TL by smoking status (Model 2), TL by BMI (Model 3), and TL by PA (Model 4) on cfPWV (*n* = 185).

Model	Variable	*B*(*SE*)	*β*	95% CI		*p*
				LL	UL	
Model 1[§]	Age (Years)	0.107 (0.042)	0.175	0.025	0.189	***0.011***
	Sex[†]	1.079 (0.779)	0.605	-0.459	2.617	*0.168*
	Occ. Smoke[‡]	0.423 (0.247)	0.117	-0.064	0.910	*0.088*
	Reg. Smoke[‡]	0.639 (0.255)	0.184	0.136	1.142	***0.013***
	BMI	0.039 (0.013)	0.233	0.013	0.064	***0.004***
	PA	-0.105 (0.060)	-0.123	-0.223	0.013	*0.082*
	MAP	0.012 (0.008)	0.114	-0.004	0.027	*0.131*
	HR	0.015 (0.006)	0.168	0.002	0.028	***0.020***
	CRP	0.025 (0.130)	0.016	-0.231	0.281	*0.848*
	TL	-0.208 (0.198)	-0.094	-0.599	0.183	*0.295*
	TL*Sex	-0.373 (0.297)	-0.564	-0.958	0.213	*0.210*
Model 2[§]	Age (Years)	0.094 (0.041)	0.154	0.013	0.176	***0.024***
	Sex[†]	0.150 (0.135)	0.084	-0.115	0.416	*0.266*
	Occ. Smoke[‡]	3.654 (1.676)	1.014	0.346	6.962	***0.031***
	Reg. Smoke[‡]	3.560 (1.450)	1.026	0.697	6.423	***0.015***
	BMI	0.041 (0.013)	0.250	0.015	0.067	***0.002***
	PA	-0.104 (0.059)	-0.122	-0.221	0.013	*0.081*
	MAP	0.010 (0.008)	0.100	-0.005	0.025	*0.179*
	HR	0.015 (0.006)	0.171	0.003	0.028	***0.016***
	CRP	0.019 (0.130)	0.012	-0.237	0.276	*0.882*
	TL	-0.216 (0.156)	-0.098	-0.524	0.091	*0.167*
	TL*Occ. Smoke	-1.299 (0.666)	-0.907	-2.614	0.016	*0.053*
	TL*Reg. Smoke	-1.092 (0.521)	-0.869	-2.120	-0.064	***0.038***

Model 3§	Age (Years)	0.110 (0.041)	0.180	0.029	0.192	***0.008***
	Sex†	0.089 (0.135)	0.050	-0.177	0.356	*0.509*
	Occ. Smoke‡	0.368 (0.245)	0.102	-0.117	0.852	*0.136*
	Reg. Smoke‡	0.697 (0.255)	0.201	0.195	1.200	***0.007***
	BMI	0.208 (0.079)	1.260	0.053	0.363	***0.009***
	PA	-0.108 (0.059)	-0.127	-0.225	0.009	*0.070*
	MAP	0.015 (0.008)	0.145	-0.001	0.030	*0.059*
	HR	0.013 (0.006)	0.141	-0.001	0.026	*0.051*
	CRP	0.051 (0.128)	0.032	-0.202	0.304	*0.692*
	TL	1.320 (0.785)	0.596	-0.230	2.870	*0.095*
	TL*BMI	-0.067 (0.030)	-1.312	-0.127	-0.007	***0.029***
Model 4§	Age (Years)	0.095 (0.042)	0.155	0.012	0.178	***0.025***
	Sex†	0.131 (0.135)	0.073	-0.137	0.398	*0.336*
	Occ. Smoke‡	0.437 (0.246)	0.121	-0.048	0.922	*0.077*
	Reg. Smoke‡	0.623 (0.252)	0.179	0.126	1.120	***0.014***
	BMI	0.038 (0.013)	0.231	0.013	0.064	***0.004***
	PA	0.601 (0.391)	0.705	-0.171	1.373	*0.126*
	MAP	0.010 (0.008)	0.097	-0.005	0.025	*0.201*
	HR	0.015 (0.006)	0.165	0.002	0.027	***0.021***
	CRP	0.039 (0.129)	0.025	-0.214	0.293	*0.759*
	TL	2.008 (1.319)	0.906	-0.596	4.612	*0.130*
	TL*PA	-0.278 (0.153)	-1.405	-0.579	0.024	*0.071*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed C-reactive protein (ng/mL); TL, log-transformed absolute telomere length (kb per diploid cell); TL*Sex, TL by sex interaction term; TL*Occ. Smoke, TL by occasional smoking interaction term; TL*Reg. Smoke, TL by regular smoking interaction term; TL*BMI, TL by BMI interaction term; TL*PA, TL by PA interaction term. *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model 1 = 0.259, $p < 0.001$; R^2 of Model 2 = 0.284, $p < 0.001$; R^2 of Model 3 = 0.272, $p < 0.001$; R^2 of Model 4 = 0.266, $p < 0.001$.

Table 15. Interaction effects of mtDNAcn by sex (Model 1), mtDNAcn by smoking status (Model 2), mtDNAcn by BMI (Model 3), and mtDNAcn by PA (Model 4) on cfPWV (*n* = 185).

Model	Variable	*B*(*SE*)	*β*	95% CI		*p*
				LL	UL	
Model 1[§]	Age (Years)	0.104 (0.041)	0.170	0.023	0.186	***0.013***
	Sex[†]	2.439 (0.181)	1.367	0.107	4.771	***0.040***
	Occ. Smoke[‡]	0.540 (0.246)	0.150	0.054	1.026	***0.030***
	Reg. Smoke[‡]	0.791 (0.262)	0.228	0.274	1.307	***0.003***
	BMI	0.037 (0.013)	0.221	0.011	0.062	***0.005***
	PA	-0.096 (0.060)	-0.112	-0.214	0.022	*0.111*
	MAP	0.014 (0.008)	0.133	-0.002	0.029	*0.079*
	HR	0.017 (0.006)	0.188	0.004	0.030	***0.008***
	CRP	0.016 (0.129)	0.010	-0.238	0.271	*0.901*
	mtDNAcn	-0.163 (0.282)	-0.051	-0.720	0.394	*0.564*
	mtDNAcn*Sex	-0.874 (0.438)	-1.351	-1.738	0.011	*0.074*
Model 2[§]	Age (Years)	0.094 (0.042)	0.153	0.012	0.176	***0.025***
	Sex[†]	0.133 (0.136)	0.074	-0.135	0.400	*0.329*
	Occ. Smoke[‡]	3.852 (2.643)	1.069	-1.365	9.070	*0.147*
	Reg. Smoke[‡]	6.732 (2.644)	1.940	1.512	11.951	***0.012***
	BMI	0.042 (0.013)	0.253	0.016	0.068	***0.002***
	PA	-0.103 (0.060)	-0.121	-0.221	0.015	*0.086*
	MAP	0.012 (0.008)	0.119	-0.003	0.027	*0.113*
	HR	0.017 (0.006)	0.188	0.004	0.029	***0.008***
	CRP	0.009 (0.129)	0.005	-0.246	0.263	*0.947*
	mtDNAcn	-0.334 (0.228)	-0.104	-0.785	0.117	*0.146*
	mtDNAcn*OS	-1.258 (0.978)	-0.946	-3.187	0.672	*0.200*
	mtDNAcn*RS	-2.085 (0.903)	-1.758	-3.868	-0.301	***0.022***

Model 3§	Age (Years)	0.104 (0.042)	0.171	0.022	0.187	***0.014***
	Sex†	0.086 (0.137)	0.048	-0.183	0.356	*0.528*
	Occ. Smoke‡	0.467 (0.247)	0.130	-0.021	0.954	*0.060*
	Reg. Smoke‡	0.705 (0.262)	0.203	0.189	1.222	***0.008***
	BMI	0.110 (0.123)	0.665	-0.134	0.354	*0.375*
	PA	-0.090 (0.061)	-0.106	-0.210	0.029	*0.138*
	MAP	0.013 (0.008)	0.125	-0.003	0.028	*0.102*
	HR	0.016 (0.006)	0.178	0.003	0.029	***0.014***
	CRP	0.033 (0.130)	0.021	-0.224	0.289	*0.802*
	mtDNAcn	0.163 (1.174)	0.051	-2.154	2.480	*0.890*
	mtDNAcn*BMI	-0.027 (0.045)	-0.493	-0.116	0.062	*0.551*
Model 4§	Age (Years)	0.100 (0.042)	0.163	0.016	0.183	***0.019***
	Sex†	0.099 (0.136)	0.056	-0.169	0.368	*0.466*
	Occ. Smoke‡	0.474 (0.246)	0.132	-0.010	0.963	*0.055*
	Reg. Smoke‡	0.720 (0.262)	0.208	0.203	1.237	***0.007***
	BMI	0.038 (0.013)	0.230	0.012	0.064	***0.004***
	PA	0.451 (0.615)	0.530	-0.763	1.665	*0.464*
	MAP	0.012 (0.008)	0.119	-0.003	0.028	*0.117*
	HR	0.017 (0.006)	0.185	0.004	0.029	***0.010***
	CRP	0.029 (0.130)	0.019	-0.227	0.286	*0.822*
	mtDNAcn	1.260 (2.019)	0.393	-2.725	5.246	*0.533*
	mtDNAcn*PA	-0.205 (0.231)	-0.914	-0.661	0.250	*0.375*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum CRP concentration (ng/mL); mtDNAcn, log-transformed mitochondrial DNA copy number (copies per diploid cell); mtDNAcn*Sex, mtDNAcn by sex interaction term; mtDNAcn*OS, mtDNAcn by occasional smoking interaction term; mtDNAcn*RS, mtDNAcn by regular smoking interaction term; mtDNAcn*BMI, mtDNAcn by BMI interaction term; mtDNAcn*PA, mtDNAcn by PA interaction term. *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model 1 = 0.266, $p < 0.001$; R^2 of Model 2 = 0.277, $p < 0.001$; R^2 of Model 3 = 0.251, $p < 0.001$; R^2 of Model 4 = 0.253, $p < 0.001$.

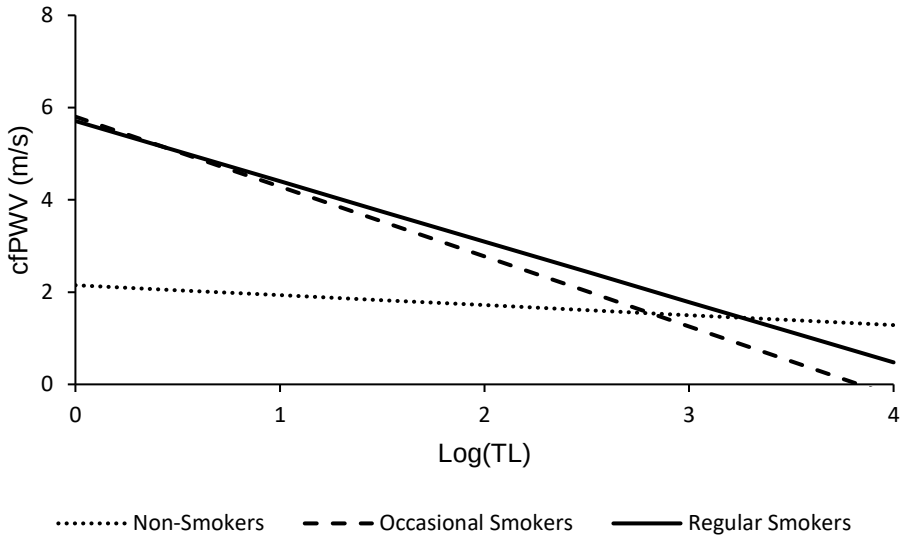

Figure 26. Interaction between log-transformed telomere length [log(TL)] and smoking status in predicting carotid-femoral pulse wave velocity (cfPWV).

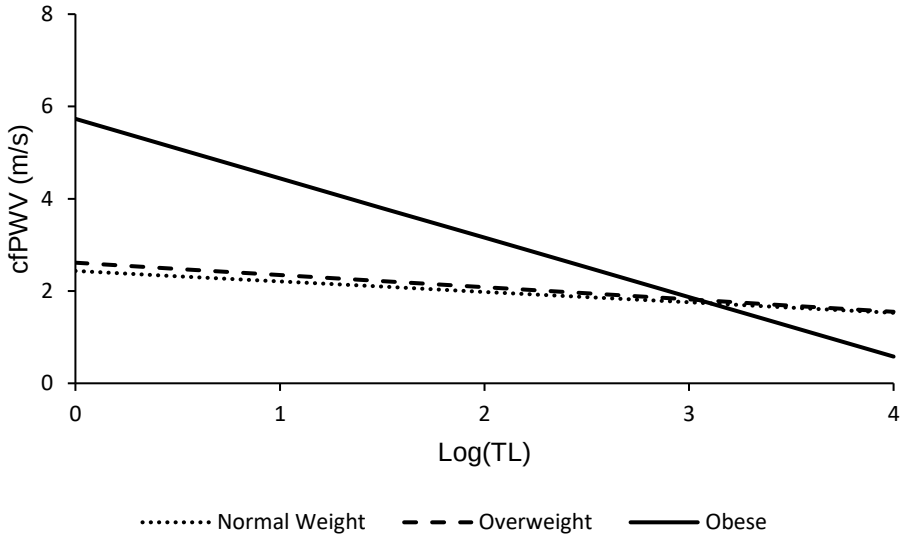

Figure 27. Interaction between log-transformed telomere length [log(TL)] and body mass index (BMI) classification in predicting carotid-femoral pulse wave velocity (cfPWV).

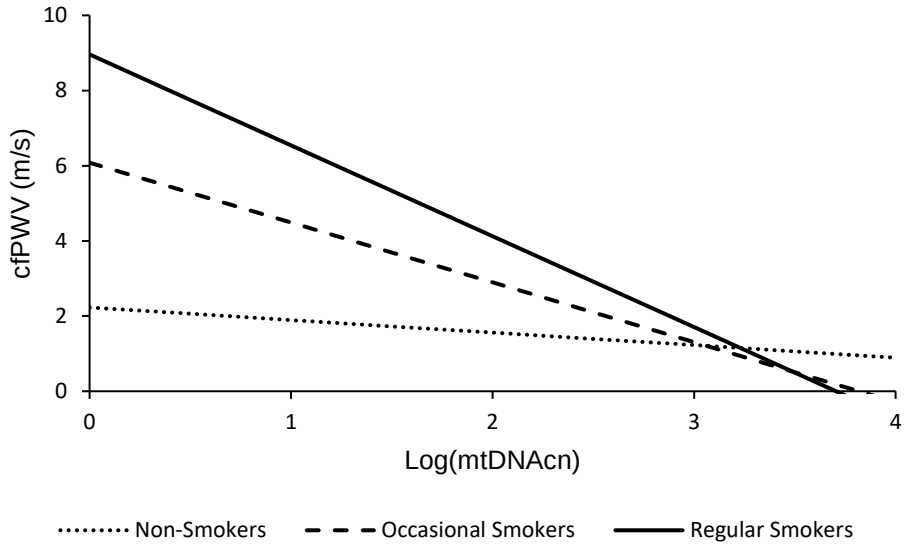

Figure 28. Interaction between log-transformed mitochondrial DNA copy number [log(mtDNAcn)] and smoking status in predicting carotid-femoral pulse wave velocity (cfPWV).

4.5 Moderation Analyses of TL and mtDNAcn on the Association between ACEs and cfPWV

The potential moderating effects of TL and mtDNAcn on the association between ACEs and cfPWV were explored. For this purpose, the interaction terms ACEs by TL (Model 1) and ACEs by mtDNAcn (Model 2) were included in subsequent regression models (Table 16). There was no significant interaction between ACEs and TL (β = 0.195, p = 0.646) nor ACEs and mtDNAcn (β = 0.583, p = 0.583) in predicting cfPWV. This suggests that neither TL nor mtDNAcn moderated the association between ACEs and cfPWV.

Table 16. Interaction effects of ACEs by TL (Model 1) and ACEs by mtDNAcn (Model 2) on cfPWV (*n* = 185).

Model	Variable	*B*(*SE*)	*β*	95% CI		*p*
				LL	UL	
Model 1§	Age (Years)	0.104 (0.041)	0.170	0.022	0.186	***0.013***
	Sex†	0.120 (0.136)	0.067	-0.148	0.388	*0.377*
	Occ. Smoke‡	0.387 (0.246)	0.107	-0.098	0.872	*0.117*
	Reg. Smoke‡	0.506 (0.257)	0.146	-0.001	1.014	***0.050***
	BMI	0.037 (0.013)	0.221	0.011	0.062	***0.005***
	PA	-0.112 (0.060)	-0.131	-0.230	0.007	*0.064*
	MAP	0.013 (0.008)	0.126	-0.002	0.028	*0.097*
	HR	0.014 (0.006)	0.158	0.002	0.027	***0.027***
	CRP	0.018 (0.129)	0.011	-0.237	0.272	*0.891*
	TL	-0.455 (0.240)	-0.205	-0.929	0.019	*0.060*
	ACEs	-0.030 (0.257)	-0.049	-0.538	0.478	*0.908*
	ACEs*TL	0.045 (0.097)	0.195	-0.146	0.235	*0.646*
Model 2§	Age (Years)	0.101 (0.042)	0.165	0.019	0.183	***0.016***
	Sex†	0.111 (0.135)	0.062	-0.156	0.378	*0.414*
	Occ. Smoke‡	0.443 (0.245)	0.123	-0.041	0.927	*0.072*
	Reg. Smoke‡	0.576 (0.261)	0.166	0.061	1.091	***0.029***
	BMI	0.035 (0.013)	0.214	0.010	0.061	***0.007***
	PA	-0.105 (0.060)	-0.123	-0.224	0.014	*0.083*
	MAP	0.013 (0.008)	0.130	-0.002	0.029	*0.086*
	HR	0.016 (0.006)	0.172	0.003	0.028	***0.017***
	CRP	0.017 (0.129)	0.011	-0.238	0.271	*0.898*
	mtDNAcn	-0.661 (0.350)	-0.206	-1.351	0.029	*0.061*
	ACEs	-0.115 (0.368)	-0.187	-0.841	0.611	*0.755*
	ACEs*mtDNAcn	0.074 (0.134)	0.333	-0.191	0.338	*0.583*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed serum C-reactive protein (ng/mL); TL, log-transformed absolute telomere length (kb per diploid cell); mtDNAcn, log-transformed mitochondrial DNA copy number (copies per diploid cell); ACEs*TL, ACEs by TL interaction term; ACEs*mtDNAcn, ACEs by mtDNAcn interaction term. *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model 1 = 0.271, $p < 0.001$; R^2 of Model 2 = 0.268, $p < 0.001$.

4.6 Mediation Analyses of TL and mtDNAcn on the Association between ACEs and cfPWV

Finally, the potential mediating effects of TL and mtDNAcn on the association between ACEs and cfPWV were explored. For this purpose, mediation analyses were performed as previously described (Baron and Kenny, 1986). In an unadjusted model, ACEs (independent variable) were significantly positively associated with cfPWV (dependent variable) (β = 0.201, p = 0.006). Additionally, in an unadjusted model, TL was significantly negatively associated with cfPWV (β = -0.176, p = 0.016). Next, it was found that in an unadjusted model, ACEs were not significantly associated with TL (mediator variable) (β = -0.014, p = 0.846). In a final regression model, both ACEs and TL were included as predictors of cfPWV. In this model, both ACEs (β = 0.198, p = 0.006) and TL (β = -0.173, p = 0.016) were significant predictors of cfPWV. Taken together, TL did not mediate the association between ACEs and cfPWV, but instead, acted as an additional variable that independently predicted cfPWV (Figure 29). This process was repeated examining mtDNAcn as the mediating variable between ACEs and cfPWV.

ACEs were significantly positively associated with cfPWV in an unadjusted model (β = 0.201, p = 0.006). In contrast, in an unadjusted model, mtDNAcn was not associated with cfPWV (β = -0.146, p = 0.054). In an unadjusted model, ACEs were not significantly associated with mtDNAcn (β = 0.017, p = 0.820). In a final regression model, both ACEs and mtDNAcn were included as predictors of cfPWV. In this model, both ACEs (β = 0.203, p = 0.005) and mtDNAcn (β = -0.145, p = 0.045) were significant predictors of cfPWV. Taken together, mtDNAcn did not mediate the association between ACEs and cfPWV, but similar to TL, acted as an additional variable that independently predicted cfPWV (Figure 30).

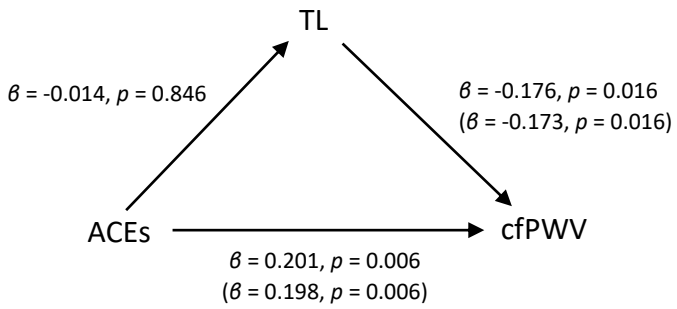

Figure 29. Mediation model examining the potential mediating effect of TL on the association between ACEs and cfPWV. *β* indicates the standardized regression coefficient; $p < 0.05$ indicates significance. Values in parentheses are standardized regression coefficients obtained from the regression model including both ACEs and TL as predictors of cfPWV.

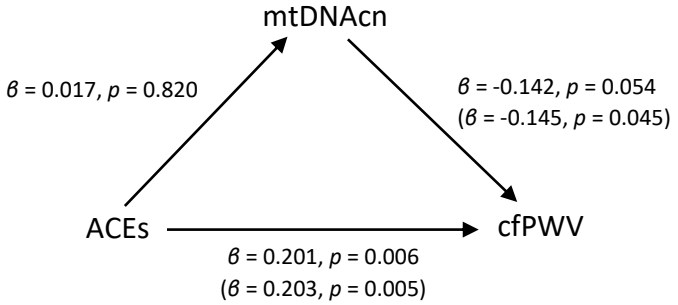

Figure 30. Mediation model examining the potential mediating effect of mtDNAcn on the association between ACEs and cfPWV. *β* indicates the standardized regression coefficient; $p < 0.05$ indicates significance. Values in parentheses are standardized regression coefficients obtained from the regression model including both ACEs and mtDNAcn as predictors of cfPWV.

Chapter V: Discussion

5.1 Introduction

The purpose of the current study was to investigate the effects of markers of biological stress (i.e., TL and mtDNAcn) on the association between ACEs and central artery stiffness (i.e., cfPWV) in a population-level sample of healthy young adults. Childhood adversity has been shown to have lasting impacts on CV health well into adulthood (Danese et al., 2009; Dong et al., 2004). One mechanism by which ACEs may influence CV health is through their association with central artery stiffness, which is assumed to be at the core of several CVDs (Nichols & O'Rourke, 2005). Pathways linking ACEs to central artery stiffness have not yet been elucidated; however, it has been postulated that dysregulation of the stress response systems may be a critical intermediary (i.e., HPA axis, SNS, and immune system) (Danese & McEwen, 2012; Horn et al., 2019). Dysregulation of these systems can result in biological stress via downstream metabolic and inflammatory pathways, which may be reflected in reductions in TL and/or mtDNAcn (Ridout et al., 2019; Tyrka et al., 2015), and may ultimately contribute to central artery stiffness (Guzik & Touyz, 2017; McEniery & Wilkinson, 2005; Zhou et al., 2012).

Telomere and mitochondrial dysfunction have been implicated in the process of biological aging (Sahin et al., 2011; Sahin & DePinho, 2012) and may reflect one mechanism by which ACEs contribute to deleterious CV health outcomes. Vascular aging, an important aspect of biological aging, is characterized by a marked increase in central artery stiffness (Laurent et al., 2019; Nilsson, 2008). To date, few studies have examined the association between childhood adversity and arterial stiffness (Klassen et al., 2016; Rafiq et al., 2020; Su et al., 2014), and none have examined the association between childhood adversity and central artery stiffness. Furthermore, the mechanisms by which ACEs contribute to arterial stiffness have not yet been fully elucidated. Markers of biological stress, such as TL and mtDNAcn, have been independently associated with both ACEs (Ridout et al., 2019; Tyrka et al., 2015) and central artery

stiffness (Benetos et al., 2001; McDonnell et al., 2017) and thus, may provide a biological link between ACEs and CV health outcomes.

In order to examine the potential effects of markers of biological stress on the association between ACEs and central artery stiffness, the current study tested several hypotheses. First, individuals who had reported experiencing a greater number of ACEs would present with a faster cfPWV and thus, a greater degree of central artery stiffness. Second, individuals who had reported experiencing a greater number of ACEs would have higher levels of biological stress, presenting as either shorter mean TL and/or lower mean mtDNAcn. Third, those presenting with a faster cfPWV would have higher levels of biological stress, presenting as either shorter mean TL and/or lower mean mtDNAcn. Fourth, the association between ACEs and cfPWV would be mediated by markers of biological stress (i.e., TL and/or mtDNAcn). Exploratory analyses were also performed in order to examine the potential moderating effects of TL and/or mtDNAcn on the association between ACEs and cfPWV.

5.2 Main Findings

The current study revealed a significant positive association between ACEs and cfPWV in a sample of healthy young adults. This association remained significant after adjustment for age, sex, smoking status, BMI, PA, instantaneous MAP and HR, and serum CRP. Interaction analyses revealed a significant interaction between ACEs and smoking, and ACEs and BMI predicting cfPWV. The current study also revealed a significant negative association between TL and cfPWV, independent of age, sex, smoking status, BMI, PA, MAP, HR, and serum CRP. In contrast, there was no significant association between mtDNAcn and cfPWV in bivariate analysis; however, after adjustment for covariates, mtDNAcn was significantly negatively associated with cfPWV. Interaction analyses revealed a significant interaction between TL and smoking, TL and BMI, and mtDNAcn and smoking in predicting cfPWV. Finally, neither TL nor mtDNAcn were associated with ACEs in the current study. Additionally, these markers of biological stress neither mediated nor moderated the association between ACEs and cfPWV.

5.3 Discussion

It has been well-established through large population-based longitudinal studies that CVD risk begins to develop and accumulate in childhood and adolescence and culminates in disease later in life (Dwyer et al., 2013). Indeed, these studies have found evidence that CVD risk factors, including obesity, dyslipidemia, and high BP during childhood predict CVD risk in adulthood (Berenson et al., 1998; Juhola et al., 2011; Li et al., 2004; Thompson et al., 2007). Interestingly, these same studies have found that exposures to childhood adversity also predict CVD risk in adulthood (Danese et al., 2009; Juonala et al., 2011; Korkeila et al., 2010). The ACE Study was the first to demonstrate a significant association between ACEs exposures and deleterious CV health outcomes in adulthood (Dong et al., 2004; Felitti et al., 1998). These initial findings continue to be bolstered by a growing body of literature demonstrating the long-term effects of ACEs on CV health and disease, with several reports demonstrating significant associations between ACEs and an increased risk of CVD (Basu et al., 2017; Su et al., 2015; Suglia et al., 2018). Indeed, ACEs have been associated with high BP and hypertension (Gooding et al., 2016; Stein et al., 2012; Su et al., 2015), and indices of atherosclerosis and arterial stiffness (Hakulinen et al., 2016; Klassen et al., 2016; Loucks et al., 2014; Rafiq et al., 2020; Su et al., 2014).

It has been postulated that ACEs (as well other CVD risk factors in childhood) may contribute to the development of CVD in adulthood through their impact on the structure and function of the central arteries (Nichols & O'Rourke, 2005; Nilsson, 2008). Central artery stiffness has emerged as one of the most important determinants of increased CVD risk (Laurent et al., 2006; Nichols & O'Rourke, 2005). In the last decade, several indices of arterial stiffness have been shown to be associated with childhood adversity (Klassen et al., 2016; Rafiq et al., 2020; Su et al., 2014). For example, Klassen et al. (2016) found that adolescent males aged 10-14 years who had experienced four or more ACEs had greater systemic arterial stiffness compared to males who had experienced less than four ACEs, independent of age, sex, SBP, HR, BMI, height, PA, family history of hypertension, and family education—a finding that

was not replicated in females of the same age stratum (Klassen et al., 2016). Similarly, Su et al. (2014) found that in healthy adolescents and young adults aged 13-29 years, those who had experienced two or more ACEs had greater peripheral arterial stiffness compared to those who had experienced less than two ACEs, independent of age, sex, ethnicity, BMI, and father's education (Su et al., 2014). Finally, in a recently published study, Rafiq et al. (2020) found that individuals who had experienced four or more ACEs had a greater increase in systemic arterial stiffness compared to those who had experienced less than four ACEs over a 9-year period from childhood (mean age 12 ± 1 years) to young adulthood (mean age 21 ± 1 years) (Rafiq et al., 2020). Importantly, this increase in systemic arterial stiffness was similar in both males and females and remained significant after adjustment for sex and changes in HR, SBP, BMI, and PA (Rafiq et al., 2020). The results of the current study are in agreement with those of the aforementioned studies. Indeed, it was found that ACEs were significantly positively associated with cfPWV, independent of age, sex, smoking status, BMI, PA, instantaneous HR and MAP, and serum CRP in a sample of healthy young adults. Together, these studies provide some evidence to suggest that the vasculature may be susceptible, in part, to childhood adversity-associated arterial stiffening.

Although the exact mechanisms linking childhood adversity to deleterious CV health outcomes have not yet been fully elucidated, at least three mechanistic pathways have been commonly identified to explain this association: behavioural, mental health, and biological pathways (Su et al., 2015; Suglia et al., 2018). With respect to biological pathways, mounting evidence suggests that childhood adversity contributes to a maladaptive stress response, which, in turn, may underlie the development of deleterious CV health trajectories throughout the lifespan (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018). Indeed, maladaptive metabolic and immune responses have been implicated as potential mediators underpinning the ACEs-CV health relationship (Horn et al., 2019; Murphy et al., 2017). It has been hypothesized that childhood adversity disrupts allostasis, resulting in physiological dysfunction across the stress response systems (Horn et al., 2019; Murphy et al., 2017; Ridout et al., 2018).

Dysfunction of these systems can result in biological stress (i.e., oxidative stress and inflammation) via downstream metabolic and inflammatory pathways, which may ultimately culminate in deleterious CV health outcomes, such as central artery stiffness and CVD (Guzik & Touyz, 2017; McEniery & Wilkinson, 2005; Zhou et al., 2012). In the current study, two markers of biological stress (i.e., TL and mtDNAcn) were examined as factors influencing the association between childhood adversity and central artery stiffness. Here, it was found that ACEs were not associated with either TL or mtDNAcn. These findings contradicted those of several previous reports (Kiecolt-Glaser et al., 2012; Puterman et al., 2016; Tyrka et al., 2010, 2016), but were in agreement with several others (Glass, 2010; Blom et al., 2015; Jodczyk et al., 2014; van Ockenburg et al., 2015; Verhoeven et al., 2015). A potential reason for the null association between ACEs and TL in the current study, as well as the heterogeneity across study findings, may be that TL does not provide a sensitive measure of ACE-associated biological stress in young adulthood (Jodczyk et al., 2014). Meta-analyses that have described the association between ACEs and TL have reported an overall negative association with aggregated small effect sizes (Hanssen et al., 2017; Li et al., 2017; Ridout et al., 2018) or no association at all (Pepper et al., 2018). Moreover, these meta-analyses have consistently reported significant heterogeneity across study findings (Hanssen et al., 2017; Li et al., 2017; Ridout et al., 2018). The diversity in study approaches, sample sizes, age of participants, measures of childhood adversity, and the method of TL measurement have been identified as potential reasons for this heterogeneity (Hanssen et al., 2017; Jodczyk et al., 2014; Li et al., 2017; Ridout et al., 2018).

Additionally, the regulation of TL is considered to be a dynamic process with several telomere-eroding and telomere-protective factors (Aviv et al., 2018; Entringer et al., 2018; Gorenjak et al., 2019). Some of these factors may modulate the association between ACEs and TL, such that the reported findings are influenced by some unknown variable (Willis et al., 2019). For example, various psychological (e.g., positive affect and resiliency) (Connolly et al., 2018; Puterman et al., 2013; Schutte et

al., 2016) and biological factors (e.g., telomerase activity) (Blackburn et al., 1989) have been shown to influence TL. These factors may modulate the association between ACEs and TL by directly influencing biological processes that contribute to telomere lengthening or indirectly by reducing an individual's susceptibility to the attritional effects of stress and adversity (Connolly et al., 2018; Puterman et al., 2013; Schutte et al., 2016).

An additional factor that may influence the association between ACEs and TL is how an ACE is perceived (i.e., the level of stress or allostatic load associated with a given ACE) (Danese & McEwen, 2012). Indeed, one meta-analysis revealed a significant, albeit small, negative association between perceived stress and TL (Mathur et al., 2016). This supports the notion of a subjective component to the purported association between ACEs and TL, such that 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 associations between ACEs and TL (Mathur et al., 2016; Willis et al., 2019). Indeed, both Verhoeven et al. (2015) and van Ockenburg et al. (2015) found recent stressful life events (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 (mean age 41.8 ± 13.1 years and 53.1 ± 11.4 years, respectively) (van Ockenburg et al., 2015; Verhoeven et al., 2015). Further, Willis et al. (2019) found the association between childhood adversity and reduced TL to be fully mediated by stressful life events in adulthood in a large sample of healthy older adults (n = 5754; mean age 69.3 ± 10.3 years) (Willis et al., 2019). Together, these studies provide evidence that the deleterious effects of stress and adversity on TL may be more marked with a shorter temporal proximity between stress exposure and TL measurement (Bürgin et al., 2019; Hanssen et al., 2017; Ridout et al., 2018). Moreover, these studies suggest that TL can recover over time (van Ockenburg et al., 2015; Verhoeven et al., 2015). Thus, future studies should adjust for recent life stress, as excluding this factor may overestimate the direct effect of ACEs on TL (Willis et al., 2019).

Similar to TL, mtDNAcn was not associated with ACEs in the current study. This finding was in direct contrast to two previous studies that found a positive association between mtDNAcn and ACEs (Ridout et al., 2019; Tyrka et al., 2016). Importantly, the current study hypothesized that an increasing number of ACEs would be associated with a reduction in mtDNAcn, which was also contradictory to the findings of Tyrka et al. and Ridout et al. However, neither Tyrka et al. nor Ridout et al. examined the long-term effects of an increasingly adverse childhood environment (i.e., the impact of an accumulation of ACEs) on mtDNAcn, which may be more consistent with the development of disease (Malik & Czajka, 2013; Siasos et al., 2018; Yue et al., 2018). Additionally, it has been postulated that mitochondria respond differently to acute versus chronic/persistent stress, such that acute stress may result in a compensatory increase in mtDNAcn, whereas chronic/persistent stress may result in a reduction in mtDNAcn and disease (Malik & Czajka, 2013; Siasos et al., 2018; Yue et al., 2018). Thus, it was hypothesized that ACEs would be negatively associated with mtDNAcn, and that mtDNAcn would mediate the interaction between ACEs and cfPWV (i.e., central artery stiffness).

In the first study to examine this association, Tyrka et al. (2016) found that those exposed to childhood maltreatment had a significantly higher mtDNAcn compared to those who were not exposed to childhood maltreatment, independent of age, sex, BMI, education, and childhood socioeconomic status, in a sample of healthy young adults (n = 290; mean age 31.0 ± 10.7 years) (Tyrka et al., 2016). Similarly, Ridout et al. (2019) found a significant positive association between mtDNAcn and childhood adversity in a sample of preschool-aged children (n = 256; mean age 4.3 ± 0.7 years) with substantiated cases of childhood maltreatment, independent of age and ethnicity (Ridout et al., 2019). The exact reasons for the contradictive findings of the current study are unclear; however, it may be due to differences in study approach, age of participants, measures of childhood adversity, or method of mtDNAcn measurement.

For example, mtDNAcn was measured 6-months post-ACE exposure in the study by Ridout et al., whereas mtDNAcn was measured at least 1-year post-ACE exposure in the participants of the current study. Similar to TL (van Ockenburg et al., 2015; Verhoeven et al., 2015; Willis et al., 2019), it may be that recent stress and adversity are more closely associated with alterations in mtDNAcn and that with the passage of time, mtDNAcn can recover (Malik & Czajka, 2013). This would be consistent with the mtDNAcn hypothesis proposed by Malik and Czajka (2013), which states that mtDNAcn increases (due to increased mitochondrial biogenesis) in response to acute stress as an adaptive response to meet the energy demands required to respond to the stressor. Removal of the stressor or a successful stress response would see mtDNAcn recover (Malik & Czajka, 2013). With respect to the current study, the temporal proximity of ACEs to mtDNAcn measurement may have nullified any impact that childhood adversity may have had on this marker of biological stress.

Additionally, the difference in participant age may explain the contradictory findings of the current study with those of Ridout et al. Indeed, Ridout et al. examined the association between mtDNAcn and ACEs in preschool-aged children (Ridout et al., 2019), whereas the current study examined the association between mtDNAcn and ACEs in a sample of healthy young adults (mean age 22.5 ± 1.5 years). Preschool-age represents a period of significant growth. Similar to TL (Frenck et al., 1998; Zeichner et al., 1999), it would be unsurprising to observe a rapid increase in mtDNAcn (due to increased mitochondrial biogenesis) during this growth period due to the energy demands associated with the high rate of cellular proliferation (Antico Arciuch et al., 2012). In contrast, mtDNAcn remains relatively stable throughout young- and middle-adulthood, and slightly declines after the age of 50 years (Knez et al., 2015; Mengel-From et al., 2014). 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 (Knez et al., 2015; Mengel-From et al., 2014; Révész et al.,

2018). Thus, age may modulate the purported association between childhood adversity and mtDNAcn, and this may partly explain differences across study findings.

Although the proximity of childhood adversity to mtDNAcn measurement and participant age may explain the contradictory findings of the current study with those of Ridout et al., they do not explain the contradictory findings of the current study with those of Tyrka et al. Similar to the current study, Tyrka et al. examined the association between ACEs and mtDNAcn in a sample of heathy adults (Tyrka et al., 2016). However, one major methodological difference between the current study and that of Tyrka et al. was the source tissue used for the assessment of mtDNAcn. Indeed, Tyrka et al. utilized whole blood for the measurement of mtDNAcn, whereas the current study utilized saliva. Although both source tissues contain leukocytes, whole blood samples additionally contain platelets, whereas saliva samples additionally contain buccal epithelial cells. To date, no study has compared saliva mtDNAcn with that of blood and thus, it remains unclear if saliva mtDNAcn is representative of mtDNAcn in other tissues. Interestingly, Knez et al. (2015) observed a significant influence of platelet and leukocyte count on whole blood mtDNAcn, such that mtDNAcn was positively associated with platelet count and negatively associated with leukocyte count (Knez et al., 2015). Accordingly, higher platelet counts in whole blood samples may lead to an overestimation of mtDNAcn, whereas higher leukocyte counts may lead to an underestimation of mtDNAcn (Hurtado-Roca et al., 2016; Knez et al., 2015). The 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.

The null association between ACEs and both TL and mtDNAcn in the current study suggest that these markers may not provide an adequate index of ACE-associated biological stress, and that TL and mtDNAcn likely do not represent a biological link between ACEs and central artery stiffness. This was supported in the current study that found that neither TL nor mtDNAcn influenced the association between ACEs and cfPWV, but instead, acted as additional variables that independently predicted

cfPWV. Future studies should aim to examine alternative pathways linking childhood adversity and arterial stiffness, such as behavioural (e.g., smoking or BMI) or mental health (e.g., PTSD) pathways, or alternative biological pathways (e.g., inflammation) that may have better utility as markers of a maladaptive stress response and ACE-associated allostatic load. Interestingly, the current study found significant interactions between ACEs and regular smoking, and ACEs and BMI in predicting cfPWV, suggesting that behavioural factors influence the association between childhood adversity and central artery stiffness. Indeed, the current study revealed that the association between ACEs and cfPWV was stronger in those categorized as regular smokers and those categorized as obese (i.e., BMI ≥ 30 kg/m^2). These findings were consistent with those of previous studies that have demonstrated an association between childhood adversity and negative health behaviours, including smoking, drug/alcohol abuse, disordered eating, consumption of energy-dense foods, and obesity (Anda, 1999; Bellis et al., 2014, 2014b; Danese & Tan, 2014; Dube et al., 2004; Felitti et al., 1998). Furthermore, negative health behaviours have been suggested to represent a mechanistic pathway by which ACEs "get under the skin" and influence CV health later in life (Su et al., 2015; Suglia et al., 2018). Importantly, inflammation has been postulated to play a key role in the pathway linking childhood adversity and related negative health behaviors to CV health outcomes (Chen & Lacey, 2018; Danese et al., 2007; Lin et al., 2016; Miller et al., 2011; Raposa et al., 2014). Future studies may aim to explore health behavior-associated inflammation in the context of ACEs and arterial stiffness.

Although neither TL nor mtDNAcn influenced the association between ACEs and cfPWV, the current study found a significant association between TL and cfPWV (unadjusted and adjusted) and mtDNAcn and cfPWV (adjusted only). These findings were in agreement with several previous studies that have examined TL and mtDNAcn in the context of central artery stiffness (Benetos et al., 2001; Foote et al., 2018; McDonnell et al., 2017). Benetos et al. (2001) were the first to report a significant negative association between TL and cfPWV, independent of age and MAP, in a sample of older males (*n*

= 120; mean age 55.0 ± 1.0 years) (Benetos et al., 2001). This finding was later replicated by Wang et al. (2011) who also found a significant negative association between TL and cfPWV, independent of age, in a sample of older males (*n* = 275; mean age 57.0 ± 10.4 years) (Wang et al., 2011). Interestingly, both Strazhesko et al. (2015) (*n* = 303; mean age 51.5 ± 13.3 years) and McDonnell et al. (2017) (*n* = 422; mean age 20.0 ± 3.0 years) found that TL was a significant negative predictor of cfPWV in both males and females, independent of age and BP (McDonnell et al., 2017; Strazhesko et al., 2015). It is unclear whether telomere shortening represents a causative biomarker in the progression of CVD or rather an epiphenomenon arising from shared underlying biological processes; however, current evidence suggests both hypotheses may hold true (De Meyer et al., 2018; Yeh & Wang, 2016; Zhan & Hägg, 2019).

Most current research examining the mechanistic pathway linking TL to CVD has mainly focused on the association between TL and atherosclerotic CVD (Yeh & Wang, 2016; Zhan & Hägg, 2019); however, arterial stiffness has been cited as potentially representing an earlier step in CVD pathology as opposed to atherosclerosis (Nilsson, 2008). TL is known to decrease with increasing age and number of mitotic divisions (Blackburn, 1991; Blackburn et al., 1989). Once telomeres become critically short, cells undergo replicative senescence and apoptosis, resulting in morphological and functional changes that may result in loss of tissue function and disease (Blackburn, 1991; de Lange, 2005; Longhese, 2012). Telomere-linked replicative senescence has been hypothesized to play an important mechanistic role in the development of deleterious CV health outcomes (Martínez & Blasco, 2018; Yeh & Wang, 2016; Zhan & Hägg, 2019). Despite their post-mitotic state, senescent vascular endothelial and smooth muscle cells remain metabolically active and exhibit the upregulation of several genes, including those encoding pro-inflammatory cytokines [e.g., interleukins (IL)-1, -6, and -8], chemokines [e.g., monocyte chemoattractant protein-1 (MCP-1)], ECM remodeling factors (e.g., MMP-2 and MMP-9), and growth factors (e.g., vascular endothelial growth factor) (Watanabe et al., 2017; Yeh & Wang, 2016). Within the vasculature, this senescence-associated secretory phenotype (SASP) is associated with inflammation and

oxidative stress, which may ultimately contribute to arterial wall remodelling and arterial stiffness (Martínez & Blasco, 2018; Wang et al., 2018; Watanabe et al., 2017; Yeh & Wang, 2016). Furthermore, SASP-associated inflammation and oxidative stress may contribute to reductions in TL and telomere dysfunction, which may further drive replicative senescence and the accumulation of cells expressing a secretory phenotype (Yeh & Wang, 2016). Therefore, a vicious cycle may exist in which telomere-linked replicative senescence leads to inflammation and oxidative stress, which, in turn, exacerbates telomere attrition and contributes to arterial stiffness (Yeh & Wang, 2016). In this sense, telomere attrition would represent both a causative factor for arterial stiffness and an epiphenomenon of vascular aging, which is in accordance with current hypotheses that link TL and arterial stiffness in the context of biological stress and aging (Nilsson, 2008).

The current study also revealed a significant interaction between TL and smoking. Indeed, the current study found that smoking modulated the association between TL and cfPWV, such that the association between TL and cfPWV was significantly stronger in regular smokers compared to non-smokers. Additionally, the association between TL and cfPWV was stronger in occasional smokers compared to non-smokers; however, the interaction was only borderline significant. Smoking-induced oxidative stress and inflammation are plausible mechanisms by which smoking influences the association between TL and arterial stiffness. Indeed, the telomeric sequence is particularly susceptible to oxidative damage due to its G-rich nature (Fouquerel et al., 2019; von Zglinicki, 2002). Guanine (G) is the most easily oxidized of the nucleobases (Steenken & Jovanovic, 1997), and the telomeric sequence is a preferred site for conversion of guanine to 8-oxoguanine (8-oxoG) (Oikawa et al., 2001). The accumulation of 8-oxoG within the telomeric sequence has been shown to promote telomere attrition by directly impeding telomere replication (Coluzzi et al., 2014, 2019; Fouquerel et al., 2019). Moreover, activation of the 8-oxoguanine DNA glycosylase-1-mediated DNA base excision repair pathway (OGG1-BER) has been shown to upregulate the expression of various pro-inflammatory cytokines and

chemokines [e.g., IL-1β, tumor necrosis factor-α (TNFα), and chemokine ligand (CCL)-3] via the induction of the NF-κB pathway (Aguilera-Aguirre et al., 2014). Thus, OGG1-BER may contribute to the maintenance of systemic low-grade inflammation (Aguilera-Aguirre et al., 2014; Ba et al., 2014), which, in turn, may influence overall cell turnover and accelerate telomere attrition and replicative senescence (Kordinas et al., 2016; Shin et al., 2019). As previously stated, senescent cells remain metabolically active and exhibit a secretory phenotype (i.e., SASP) that, within the vasculature, may contribute to arterial stiffness (Martínez & Blasco, 2018; Wang et al., 2018; Watanabe et al., 2017; Yeh & Wang, 2016).

In addition to the significant interaction between smoking and TL, the current study revealed a significant interaction between BMI and TL in predicting cfPWV. Indeed, it was found that as BMI increased, the association between TL and cfPWV became stronger. Interestingly, this interaction appeared to be driven by those who were considered obese (BMI ≥ 30 kg/m^2). Similar to smoking, increasing adiposity and obesity are strongly associated with systemic low-grade inflammation pertinent to vascular remodeling and arterial stiffness (Brunner et al., 2015; García-Prieto et al., 2019; Welendorf et al., 2019). Circulating levels of several pro-inflammatory cytokines (e.g., CRP, IL-1β, IL-6, IL-8, MCP-1, and TNFα) have been shown to be increased in obesity (Lacasa et al., 2007; O'Hara et al., 2012). It has been suggested that some of these cytokines largely originate from the stromal vascular fraction, which includes infiltrated macrophages, among other cell types (Curat et al., 2006; Lacasa et al., 2007). Macrophages are an important contributor to obesity-associated inflammation (Curat et al., 2006; O'Hara et al., 2012), which, similar to smoking-induced inflammation, may influence cell turnover and accelerate telomere attrition (Welendorf et al., 2019). Telomere-linked replicative senescence and the associated secretory phenotype (i.e., SASP) may ultimately contribute to central artery stiffness (Martínez & Blasco, 2018; Wang et al., 2018; Watanabe et al., 2017; Yeh & Wang, 2016). Additionally, the enhanced production of ROS by infiltrated macrophages may contribute to the development of 8-oxoG lesions within the telomeric sequence, which, as previously described, can lead to telomere

attrition (Aguilera-Aguirre et al., 2014) and, more distally, arterial stiffness (Martínez & Blasco, 2018; Wang et al., 2018; Watanabe et al., 2017; Yeh & Wang, 2016).

Increasing adiposity and obesity may also influence the association between TL and cfPWV via insulin resistance (Strazhesko et al., 2015; Zhan & Hägg, 2019). Indeed, obesity-associated insulin resistance has been linked with chronic low-grade inflammation and oxidative stress, which are known to reduce TL (Strazhesko et al., 2015; von Zglinicki, 2002; Yeh & Wang, 2016). Moreover, obesity and insulin resistance have been shown to alter arterial structure and function (Herouvi et al., 2013; Jia et al., 2015), and increasing insulin resistance has been linked to shorter telomeres and a faster cfPWV in healthy middle-aged adults (n = 303; mean age 51.5 ± 13.3 years) (Strazhesko et al., 2015). Accordingly, obesity-associated insulin resistance, inflammation, and oxidative stress may have a synergistic effect on telomere attrition and central artery stiffness, which may be reflected in the stronger association between TL and cfPWV with increasing adiposity (i.e., BMI). To summarize, both obesity- and smoking-induced oxidative stress and inflammation may contribute to both a reduction in TL and an increase in central artery stiffness. Telomere shortening may further exacerbate arterial stiffening via the induction of replicative senescence and the senescence-associated secretory phenotype.

Similar to TL, mtDNAcn was significantly negatively associated with cfPWV in the current study, independent of age, sex, smoking status, BMI, PA, MAP, HR, and serum CRP. This finding was in line with other published studies that have linked mitochondrial dysfunction (Gioscia-Ryan et al., 2018; LaRocca et al., 2014) and mtDNAcn (Foote et al., 2018) with central artery stiffness in mice. Although not a direct measure of mtDNA damage or mitochondrial dysfunction, mtDNAcn is associated with mitochondrial respiration (Foote et al., 2018; Jeng et al., 2008), and may therefore serve as an indirect biomarker of mitochondrial function (Ashar et al., 2017; Yue et al., 2018). To date, no human studies have examined the association between mtDNAcn (or mitochondrial dysfunction) and arterial stiffness; however, recent murine models have revealed the importance of mitochondrial function in central artery stiffness (Foote

et al., 2018; Gioscia-Ryan et al., 2018; LaRocca et al., 2014). Indeed, LaRocca et al. (2014) found that mitochondrial dysfunction was associated with elevated ROS production and a faster cfPWV in mice (LaRocca et al., 2014). Interestingly, the authors also found that restoring mitochondrial function reduced cfPWV—a finding the authors attributed to associated reductions in ROS-mediated collagen deposition within the arterial wall ECM (LaRocca et al., 2014). In agreement with these findings, Gioscia-Ryan et al. (2018) found that treating aged mice with a mitochondria-targeted antioxidant (i.e., MitoQ) reduced their degree of aortic stiffness, which was partly attributed to antioxidant-induced attenuation of ROS-mediated elastin degradation within the aortic wall (Gioscia-Ryan et al., 2018). Finally, Foote et al. (2018) demonstrated that a reduction in mtDNAcn was associated with a reduction in mitochondrial respiration and a greater degree of central artery stiffness (i.e., a faster aortic PWV) in mice (Foote et al., 2018). Moreover, these authors found that restoring mtDNAcn improved both mitochondrial respiration and arterial stiffness (Foote et al., 2018). Together, the results of these studies, along with those of the current study, provide evidence that a reduction in mtDNAcn and thus, mitochondrial function, may be important in central artery stiffness. Specifically, alterations in mitochondrial function and/or mtDNAcn may be associated with arterial wall ECM remodelling and arterial stiffness.

The current study additionally revealed a significant interaction between mtDNAcn and smoking in predicting cfPWV, such that the association between mtDNAcn and cfPWV was stronger in regular smokers compared to non-smokers. Moreover, although there was no significant interaction between occasional smoking and mtDNAcn, the current study provided preliminary evidence of a modulatory effect of smoking intensity on the interaction between smoking and mtDNAcn in predicting cfPWV. Future studies should utilize more robust measures of smoking intensity (e.g., packs per day or pack-years) to explore the effects of smoking on the association between mtDNAcn—and TL—and cfPWV. Smoking-induced oxidative stress is a plausible mechanism by which smoking influences the association between mtDNAcn and central artery stiffness (Liu et al., 2015; Wu et al., 2019). Indeed, smoking

induces intracellular ROS production of which mitochondria are the main producers (Liu et al., 2015; Zheng et al., 2019). MtDNA is particularly susceptible to oxidative damage due to its proximity to mitochondrial ROS, lack of histone proteins, and limited DNA damage repair capacity (Cadenas & Davies, 2000; Yakes & Van Houten, 1997). To compensate for mtDNA damage and protect themselves from oxidative damage, mitochondria may upregulate the expression of antioxidant scavengers, modulate oxidative phosphorylation, exchange mtDNA through fission/fusion, or upregulate the expression of proteins regulating mitochondrial biogenesis (e.g., PGC-1α/β) (Liu et al., 2015; Wu et al., 2019). Continual exposure to smoking-induced oxidative stress, however, may surpass mitochondrial capacity to compensate for oxidative damage and ultimately lead to a reduction in mtDNAcn (Malik & Czajka, 2013; Wu et al., 2019). Thus, it is possible that smoking-induced reductions in mtDNAcn alter mitochondrial function, which, in turn, may contribute to arterial wall remodeling and central artery stiffness (Gioscia-Ryan et al., 2018; LaRocca et al., 2014).

TL and mtDNAcn were also significantly positively correlated in the current study, which is consistent with previous findings (Kim et al., 2013; Tyrka et al., 2015; Zole et al., 2018). Previous studies have linked telomeres and mitochondria at a functional level (Sahin et al., 2011; Sahin & DePinho, 2012; Zheng et al., 2019), and the "telomere-mitochondria axis" has been postulated to contribute to the development and progression of CVD (Moslehi et al., 2012; Sahin & DePinho, 2012). Indeed, reductions in TL and telomere dysfunction have been linked to the activation of the p53 protein, which, in turn, binds to the promoters of *PGC-1α* and *PGC-1β*, thereby suppressing their expression (Sahin et al., 2011; Sahin & DePinho, 2012). Suppression of *PGC-1α* and *PGC-1β* leads to reductions in both mitochondrial biogenesis and function (and subsequently mtDNAcn), as well as increased levels of ROS (Sahin et al., 2011; Sahin & DePinho, 2012). In addition to telomere-linked activation of p53, telomere dysfunction has been shown to trigger a DNA damage response, which results in the activation of the p21 protein (Passos et al., 2010). Activation of p21 can induce mitochondrial dysfunction and increased production

of ROS, which, in turn, can accelerate the onset of replicative senescence (e.g., by accelerating the rate of telomere attrition) and its associated secretory phenotype (Passos et al., 2010; von Zglinicki, 2002). mtDNAcn may serve as a useful biomarker of mitochondrial function (Malik & Czajka, 2013; Yue et al., 2018), and may also be directly linked to TL (Zole & Ranka, 2019) and vascular aging (Foote et al., 2018). Thus, telomere-linked activation of p53 and p21 have been postulated to contribute to functional decline in post-mitotic tissues via replicative senescence and mitochondrial dysfunction, which, in turn, may contribute to vascular aging and central artery stiffness.

5.4 Strengths, Limitations, and Future Directions

The current study had several strengths and limitations. To the authors' knowledge, this was the first study to explore the effects of markers of biological stress (i.e., TL and mtDNAcn) on the association between childhood adversity and central artery stiffness. Although the current study did not find a significant modulatory effect of either TL or mtDNAcn on the association between ACEs and central artery stiffness, it was revealed that these markers of biological stress, along with ACEs, independently predicted cfPWV. However, due to the cross-sectional nature of the current study, it is difficult to infer causal associations between markers of biological stress, ACEs, and central artery stiffness. Future studies should aim to examine alternative pathways linking childhood adversity and central artery stiffness, such as behavioural or mental health pathways, or alternative biological pathways that may have better utility as markers of a maladaptive stress response and ACE-associated allostatic load. Furthermore, future experimental models are needed to better understand the association between TL and mtDNAcn with central artery stiffness. Specifically, future studies should determine whether TL and mtDNAcn represent a mechanistic pathway contributing to arterial stiffness, or if reductions in TL and mtDNAcn are epiphenomena of vascular aging.

Another strength of the current study was the utilization of the gold-standard measure of arterial stiffness (i.e., cfPWV), along with robust biological measures (i.e., qPCR), and a comprehensive

questionnaire that detailed childhood adversity exposures (i.e., CTES 2.0), various health behaviors (e.g., smoking and PA level), and several other measures (e.g., mental health status and medication use) that may be pertinent to future studies. Acquisition of cfPWV is considered the most simple, non-invasive, and reproducible method of determining central artery stiffness (Laurent et al., 2006; Van Bortel et al., 2012). Additionally, cfPWV has been demonstrated to be an independent predictor of CVD within several populations (Ben-Shlomo et al., 2014; Laurent et al., 2006; Vlachopoulos et al., 2010) and, as such, has been recommended as a clinical marker of CV risk stratification (Townsend et al., 2015).

Importantly, certain aspects of cfPWV measurement can also pose limitations. For example, cfPWV is calculated by dividing the pulse travel distance (i.e., Δd) by the PTT (i.e., Δt). Measurement of the "true" pulse travel distance inside the body is difficult to assess non-invasively, as body size and measurement technique can influence surface distance (Laurent et al., 2006; Sugawara et al., 2016; Van Bortel et al., 2016). Thus, assimilating the pulse travel distance to the surface measurement can lead to inaccuracies in cfPWV calculation. The current study measured the CCA-to-femoral distance as the sum of the distance from the suprasternal notch to the umbilicus and the umbilicus to the femoral artery minus the distance from the suprasternal notch to the CCA. This method of determining distance is not the current recommendation for the non-invasive measurement of pulse travel distance in the assessment of cfPWV (Van Bortel et al., 2012). Indeed, this method has been shown to underestimate the true pulse travel distance (measured via MRI) and thus, underestimate cfPWV (Huybrechts et al., 2011; Sugawara et al., 2016). In contrast, the current recommendation of 80% CCA-to-femoral straight distance has been shown to be more accurate (Huybrechts et al., 2011; Sugawara et al., 2016). Although the current study employed a "less accurate" method to assess pulse travel distance, the use of this measurement in every participant would still allow the researchers to examine trends in the association between cfPWV, ACEs, and biological markers of stress in a population-level sample of healthy young adults. Moreover, this method of assessing pulse wave distance has been recommended by previous

expert consensus documents (Laurent et al., 2006; Van Bortel et al., 2002) and has been shown to have utility in several populations (Canepa et al., 2014; Ring et al., 2014, 2018; Rodriguez et al., 2016; Sutton-Tyrrell, 2001; Weir-McCall et al., 2018).

It has also been shown that approximately 80% of the variation in cfPWV is explained by PTT, whereas only 9-15% is explained by pulse travel distance (Sugawara et al., 2016). In the current study, PTT was determined using the foot-to-foot method, which is a well-established and commonly used technique for determining PTT (Laurent et al., 2006; Nichols & O'Rourke, 2005). The foot of the pulse waveform (i.e., the onset of the systolic upstroke of the forward pulse wave) was identified based on phase velocity theory, which suggests that the foot of the waveform is primarily composed of frequencies between 5-30 Hz (McDonald, 1968; Munakata, 2003). By filtering out the lower (≤ 5 Hz) and higher frequencies (≥ 30 Hz), the foot of the waveform was more easily identified as the minimum value of the filtered signal (Koelwyn et al., 2012; Munakata, 2003). Additionally, three consecutive sequences of at least 15 PP waveforms were obtained at both the CCA and femoral arterial sites, and 15 of the most well-defined and consistent waveforms were averaged in the calculation of PTT. Therefore, in addition to pulse travel distance, PTT was adequately measured in order to minimize any error in the calculation of cfPWV, which is a major strength of the current study.

To assess childhood adversity, the current study employed the CTES 2.0, which is a self-report questionnaire that provides information on a variety of stressful childhood experiences, including childhood maltreatment (i.e., abuse and neglect) and household dysfunction, as well as several other potentially stressful and/or traumatic events (Edwards et al., 2007; Wade et al., 2019). The diversity in ACEs examined in the current study allowed the authors to more comprehensively examine the association between ACEs, markers of biological stress, and central artery stiffness. The current study was also unique in that the study sample consisted of individuals aged 19-25 years. This may have allowed for better recall of ACEs, as the participants would have been temporally more proximal to the

time of exposure. Interestingly, at least for the purported association between ACEs and markers of biological stress (specifically TL), studies that utilize more comprehensive assessments of childhood adversity (e.g., those that examine maltreatment and household dysfunction) and studies involving younger participants (i.e., shorter duration between adversity exposure and biological measurement) typically report greater magnitudes of association compared to studies that more narrowly assess childhood adversity (e.g., examine only abuse) and those involving older participants (Bürgin et al., 2019; Li et al., 2017; Ridout et al., 2018). Thus, by utilizing a more comprehensive assessment of childhood adversity and a sample of young adults, the current study may have been well-suited to determine the "true" association between ACEs, markers of biological stress, and central artery stiffness.

A potential limitation to utilizing the CTES 2.0 is that self-report questionnaires are subject to both self-report and recall bias. In the current study, ACEs data relied on individuals' retrospective self-reports of childhood adversity, which may be considered highly sensitive and somewhat intrusive and thus, may be inherently sensitive to biases. The inclusion of young adults (aged 19-25 years) in the current study may have limited recall bias due to a shorter temporal proximity between exposure and reporting. Additionally, the CTES 2.0 was administered at the end of the laboratory visit, as well as near the end of the broader questionnaire package. At this time, the researchers had already 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 childhood maltreatment, household dysfunction, and other ACEs (Dube et al., 2004; Edwards et al., 2007). Finally, although childhood adversity can be assessed via interview (Vrshek-Schallhorn et al., 2014), a self-report questionnaire was utilized in the current study as a means of minimizing participant discomfort and social response bias (Edwards et al., 2001, 2007). Together, the utilization of the CTES 2.0, along with the environment in which it was administered and the age of participants, likely mitigated any potential biases on the current findings.

In the current study, qPCR techniques were used to assess both TL and mtDNAcn. Although qPCR is not considered the gold-standard method for the quantification of TL, it is well-suited for large epidemiological studies due to its relatively low cost and less laborious nature relative to TRF analysis (i.e., Southern blot assay) (Lai et al., 2018; Montpetit et al., 2015). However, although qPCR produces results that are correlated with TRF analysis (Aviv et al., 2011; Cawthon, 2002; O'Callaghan & Fenech, 2011), it also tends to have greater measurement error (Aviv et al., 2011; Verhulst et al., 2015), which may be considered a limitation of the current study. In contrast, qPCR is considered the gold-standard measure of mtDNAcn quantification; however, this is currently being re-evaluated as new methods of assessing mtDNAcn emerge (Longchamps et al., 2020).

The current study utilized saliva as the source tissue for DNA isolation and subsequent TL and mtDNAcn measurement, which is less commonly used in comparison to venous blood samples (Willis et al., 2019). One potential concern regarding the use of saliva samples for TL measurement is the presence of buccal epithelial cells in the sample, as TL from this cell type does not show the same negative association with age that TL from other source tissues have shown (Goldman et al., 2018; Willis et al., 2019). However, several studies have demonstrated a significant correlation between TL extracted from saliva and TL extracted from venous 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 (Goldman et al., 2018; Mitchell et al., 2014; Stout et al., 2017).

As previously described, source tissue can also influence mtDNAcn measurement. Although both saliva and venous blood samples contain leukocytes, blood samples (specifically, whole blood samples) additionally contain platelets, and saliva samples additionally contain buccal epithelial cells. To date, no study has compared saliva mtDNAcn with that of blood and thus, it remains unclear if saliva mtDNAcn is representative of mtDNAcn in other tissues. Interestingly, Knez et al. (2015) observed a significant

influence of platelet and leukocyte count on whole blood mtDNAcn, such that mtDNAcn was positively associated with platelet count and negatively associated with leukocyte count (Knez et al., 2015). Accordingly, higher platelet counts in whole blood samples may lead to an overestimation of mtDNAcn, whereas higher leukocyte counts (e.g., in infection, inflammation, or stress) may lead to an underestimation of mtDNAcn (Hurtado-Roca et al., 2016; Knez et al., 2015). Furthermore, studies have shown that both mtDNAcn and TL can vary by blood sample type [(i.e., whole blood *vs.* peripheral blood mononuclear cells (PBMCs)] (Zole & Ranka, 2019), and also leukocyte composition (Hurtado-Roca et al., 2016; Knez et al., 2015; Lin et al., 2016). Thus, due to the influence of source tissue and cellular composition, the results of the current study (as well as those of other studies measuring mtDNAcn and TL) may be inherently limited in their generalizability. Future studies may aim to replicate the findings of the current study using other source tissues for the measurement of TL and mtDNAcn.

5.5 Conclusion

The current study showed for the first time that ACEs were significantly and positively associated with cfPWV and thus, central artery stiffness. In contrast, ACEs were not associated with either TL or mtDNAcn, and neither marker moderated nor mediated the association between ACEs and cfPWV. Based on these findings, it was concluded that TL and mtDNAcn may not provide sensitive measures of ACE-associated biological stress in young adulthood, and that these markers likely do not represent a mechanistic pathway linking childhood adversity to central artery stiffness; however, future experimental studies are required to confirm these conclusions. Interestingly, the current study revealed a significant interaction effect between ACEs and smoking, and ACEs and BMI in predicting cfPWV. These findings lend support to the hypothesis that negative health behaviours provide a link between childhood adversity and deleterious CV health outcomes. Future epidemiological and experimental studies should aim to explore the role of negative health behaviours, particularly smoking and obesity, in the association between ACEs and central artery stiffness.

Additionally, the current study revealed a significant negative association between both TL and mtDNAcn with cfPWV, as well as significant interactions between both markers with smoking and BMI in predicting cfPWV. With respect to the association between TL and mtDNAcn with cfPWV, smoking and obesity may contribute to reductions in TL and mtDNAcn by inducing biological stress, which, in turn, may contribute to central artery stiffness (i.e., smoking and adiposity lie on the mechanistic pathway between TL and/or mtDNAcn and cfPWV). Conversely, smoking- and obesity-induced biological stress may independently contribute to reductions in both markers, and an increase in arterial stiffness (i.e., reductions in TL and/or mtDNAcn and increased arterial stiffness are epiphenomena of smoking- and obesity-induced biological stress). The latter hypothesis would suggest that smoking and obesity are associated with vascular aging, which has been postulated to be characterized by reductions in TL and mtDNAcn, as well as an increase in central artery stiffness. Future experimental models are needed to determine whether TL and mtDNAcn represent a mechanistic pathway contributing to central artery stiffness, or if reductions in TL and mtDNAcn are epiphenomena of vascular aging.

Contributions

Data were collected and analyzed at Brock University. Dr. Terrance Wade and Dr. Deborah O'Leary are principal investigators for the NLHS on which this thesis was based. Anthropometric and CV data were collected and analyzed by Kylie Dempster and Nathaniel Iannarelli. TL, mtDNAcn, and serum CRP analyses were performed by Jessy Moore, Nathaniel Iannarelli, Aindriu Maguire, and Madison Gagnon under the direction of Dr. Adam MacNeil. Nathaniel Iannarelli, Dr. Deborah O'Leary, Dr. Adam MacNeil, and Dr. Terrance Wade were responsible for the conceptualization and data analysis of the current work. Nathaniel Iannarelli was the author of this thesis. Dr. Deborah O'Leary, Dr. Adam MacNeil, and Dr. Terrance Wade revised the thesis document and approved the final version.

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Appendices

Appendix A: Assessment of Smoking Status

Smoking

Do you currently smoke cigarettes daily, occasionally or not at all?

<table>
<tr><th>Daily</th><th>Occasionally</th><th colspan="2">Not at all</th></tr>
<tr><td>About how many cigarettesdo you usually smoke each day now?</td><td>On the days that you smoke, about how many cigarettes do you usually smoke?</td><td colspan="2">Have you ever smoked more than 100 cigarettes in your lifetime (about 4 packs)?
Yes No</td></tr>
<tr><td rowspan="2"></td><td>About how many days in a week do you smoke cigarettes?</td><td>If yes, when did you stop smoking?</td><td>If no, Have you eversmoked a single cigarette?
Yes No</td></tr>
<tr><td>How old were you whenyou smoked your first cigarette? __</td><td>How old were you when yousmoked your first cigarette?</td><td>How old were youwhen you smokedyour first cigarette?</td><td>If yes, how old wereyou when you smoked your first cigarette?</td></tr>
</table>

Appendix B: International Physical Activity Questionnaire – Short Form (IPAQ-SF)

Health and Physical Activity

	Excellent	Very Good	Good	Fair	Poor
In general, how would you say your health is?	1	2	3	4	5
In general, how would you rate your emotional health today?	1	2	3	4	5
In general, how would you rate your physical health today?	1	2	3	4	5

We want to know about the time you spent being physically active **in the last 7 days**. Please answer each question even if you do not consider yourself to be an active person. **Think about all of the activities you do at work, as part of your house and yard work, to get from place to place, and in yourspare time for recreation, exercise or sport.**

Now, think about all the **vigorous activities which take hard physical effort** that you did in the last 7 days. **Vigorous activities** make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.

1. **During the last 7 days, on how many days did you do vigorous physical activities? (Think onlyabout those physical activities that you do for at least 10 minutes at a time.)**
   - _____Days per week

2. **How much time did you usually spend doing vigorous physical activities on one of those days?(If you are not sure, think about a specific day, for example, Wednesday?)**
   - _____Hours per day **OR** _____Minutes per day

Now, think about **activities which take moderate physical effort** that you did in the last 7 days. **Moderate physical activities** make you breathe somewhat harder than normal and may include carryinglight loads, bicycling at a regular pace, or doubles tennis. **Do not include walking**. Again, think about only those physical activities that you did for at least 10 minutes at a time.

3. **During the last 7 days, on how many days did you do moderate physical activities? (Think onlyabout those physical activities that you do for at least 10 minutes at a time.)**
   - _____Days per week

4. **How much time did you usually spend doing moderate physical activities on one of thosedays? (If you are not sure, think about a specific day, for example, Wednesday?)**
   - _____Hours per day **OR** _____Minutes per day

Now, think about the time you spent **walking** in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. **During the last 7 days, on how many days did you walk for at least 10 minutes at a time?**
   - _____Days per week

6. **How much time did you usually spend walking on one of those days? (If you are not sure,think about a specific day, for example, Wednesday?)**
   - _____Hours per day **OR** _____Minutes per day

Now, think about the time you spent **sitting** on week days during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at adesk, visiting friends, reading or sitting or lying down to watch television.

7. **During the last 7 days, how much time did you usually spend sitting on a week day? (If you arenot sure, think about a specific day, for example, Wednesday?)**
   - _____Hours per day **OR** _____Minutes per day

Appendix C: Childhood Trust Event Survey 2.0 (CTES 2.0)

Negative Childhood Experiences (CTES 2.0)

Remember that all of the information you provide will be kept strictly confidential and will be coded anonymously so that your name will never be associated with answers to any questions.

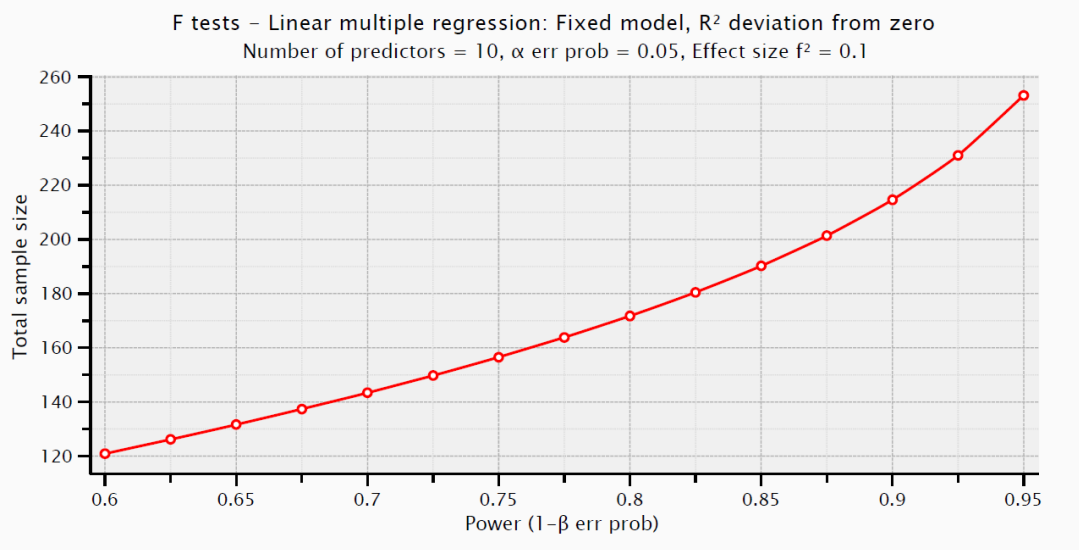
It is important for us to understand what may have happened to you in the past. The questions belowdescribe some kinds of upsetting experiences. Since we give these questions to everyone, we list a lot of possible events that may have happened at any time in your life. If you <u>have</u> experienced the eventat some time in your life, please circle Yes. If you <u>have not</u> experienced the event at some time in yourlife, please circle No.

When you were a child:		
1. Were you ever in a really bad accident, such as a serious car accident?	**Yes**	**No**
2. Were you ever in a disaster such as a tornado, hurricane, fire, big earthquake, or flood?	**Yes**	**No**
3. Were you ever so badly hurt or sick that you had to have painful or scary medical treatment?	**Yes**	**No**
4. Have you ever been threatened or really picked on by a bully (someone outside of your family)?	**Yes**	**No**
5. Have you ever had a parent swear at you, insult you, put you down, or say a bad, or "I wish you were never born"?	**Yes**	**No**
6. Were you ever completely separated from your parent(s) for a long time, such as going to a foster home, your parent living far apart from you, or never seeing your parent again?	**Yes**	**No**
7. Have you ever had a family member who was put in jail or prison or taken away by the police?	**Yes**	**No**
8. Have you ever had a time in your life when you did not have the care you needed, such as not having enough to eat, being left in charge of your younger brothers or sisters for long periods of time, or being left with a grownup who used drugs?	**Yes**	**No**
9. Have you ever had a time in your life when you were living in a car, living in a homeless shelter, living in a battered women's shelter, or living on the street?	**Yes**	**No**
10. Have you ever had someone living in your home who abused alcohol or used street drugs?	**Yes**	**No**
11. Have you ever had someone in your home try to hurt or kill himself/herself, such as cutting himself/herself or taking too many pills or drugs?	**Yes**	**No**
12. Have you ever had a family member who was depressed or mentally ill for a long time?	**Yes**	**No**
13. Have you ever had a family member or someone else very close to you die unexpectedly?	**Yes**	**No**
14. Has someone in your home ever been physically violent toward you, such as whipping, kicking, or hitting hard enough to leave marks?	**Yes**	**No**

15. Has an adult ever said they were going to hurt you really badly or kill you, or acted like they were going to hurt you very badly or ki actually do it?	**Yes**	**No**

16. Have you ever seen or heard family members act like they were going to kill or hurt each other badly, even if they didn't actually do it?	**Yes**	**No**
When you were a child:		
17. Have you ever seen or heard a family member being hit, punched, kicked very hard, or killed?	**Yes**	**No**
18. Have you ever seen someone in your neighborhood be beaten up, shot at or killed?	**Yes**	**No**
19. Has someone ever robbed or tried to rob (jump) you or your family with a weapon?	**Yes**	**No**
20. Has someone ever kidnapped you (taken you away from your home when they shouldn't have) or has someone close to you ever been kidnapped?	**Yes**	**No**
21. Have you ever been badly hurt by an animal, such as attacked by a dog?	**Yes**	**No**
22. Have you ever had a pet or animal that was hurt or killed on purpose by someone you knew?	**Yes**	**No**
23. Have you ever seen a friend killed?	**Yes**	**No**
24. Has someone ever touched your private sexual body parts when you did not want them to?	**Yes**	**No**
25. Has someone ever made you touch his or her private sexual body parts?	**Yes**	**No**
26. Has an adult ever tied you up, gagged you, blindfolded you, or locked you in a closet or a dark scary place?	**Yes**	**No**
27. Have your parents ever separated or divorced?	**Yes**	**No**
28. Have you ever saw your parents have a really serious argument or conflict?	**Yes**	**No**
29. Have you moved residences or schools?	**Yes**	**No**

Appendix D: Sample Size Calculations for Regression and Mediation Analyses



F tests – Linear multiple regression: Fixed model, R^2 deviation from zero

Analysis:	A priori: Compute required sample size		
Input:	Effect size f^2	=	0.10
	α err prob	=	0.05
	Power (1–β err prob)	=	0.80
	Number of predictors	=	10
Output:	Noncentrality parameter λ	=	17.2000000
	Critical F	=	1.8899310
	Numerator df	=	10
	Denominator df	=	161
	Total sample size	=	172
	Actual power	=	0.8007123

Figure S1. X-Y plot illustrating the total sample size required to detect a small-to-medium effect size (Cohen's f^2 = 0.10) of ACEs on cfPWV after adjusting for age, sex, BMI, PA, smoking status, MAP, HR, and serum CRP (i.e., 10 predictor variables) at an α-level of 0.05 and over a range of statistical powers $(1-\beta)$.

Supplemental Equation 1:

$$f^2 = \frac{R^2}{1 - R^2}$$

$$f^2 = \frac{0.071}{1 - 0.071}$$

$$f^2 = 0.076$$

Supplemental Equation 2:

$$n = \frac{L}{f^2} + k + 1$$

$$n = \frac{7.850}{0.076} + 1 + 1$$

$$n = 105.289$$

$$n \approx 106$$

Figure S2. Calculation determining the required sample size to detect the mediated effect of TL on the association between ACEs and cfPWV. Cohen's f^2 was calculated using Supplemental Equation 1, where R^2 is the proportion of variance in cfPWV explained by ACEs after adjustment for TL. The required sample size was calculated using Supplemental Equation 2, where *n* is the required sample size, *L* is a tabled value corresponding to a specific power value (at an α-level of 0.05 and power of 0.8, *L* is equal to 7.850), f^2 is the effect size, and *k* is the number of predictor variables.

Supplemental Equation 1:

$$f^2 = \frac{R^2}{1 - R^2}$$

$$f^2 = \frac{0.061}{1 - 0.061}$$

$$f^2 = 0.065$$

Supplemental Equation 2:

$$n = \frac{L}{f^2} + k + 1$$

$$n = \frac{7.850}{0.065} + 1 + 1$$

$$n = 122.002$$

$$n \approx 123$$

Figure S3. Calculation determining the required sample size to detect the mediated effect of mtDNAcn on the association between ACEs and cfPWV. Cohen's f^2 was calculated using Supplemental Equation 1, where R^2 is the proportion of variance in cfPWV explained by ACEs after adjustment for mtDNAcn. The required sample size was calculated using Supplemental Equation 2, where *n* is the required sample size, *L* is a tabled value corresponding to a specific power value (at an α-level of 0.05 and power of 0.8, *L* is equal to 7.850), f^2 is the effect size, and *k* is the number of predictor variables.

Appendix E: Example ΔΔCq Analysis

Primer Set	Target Sample	Reference Sample	Known Lengths (kb)
Telomere	11.56	16.30	369.00 ± 18.00
SCR	21.27	27.35	27.60 ± 0.40

$$\begin{aligned}\Delta Cq(TL) &= Cq(TL, target\ sample) - Cq(TL, reference\ sample)\\ &= 11.56 - 16.30\\ &= -4.74\end{aligned}$$

$$\begin{aligned}\Delta Cq(SCR) &= Cq(SCR, target\ sample) - Cq(SCR, reference\ sample)\\ &= 21.27 - 27.35\\ &= -6.08\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(TL) &= \Delta Cq(TL) - \Delta Cq(SCR)\\ &= -4.74 - (-6.08)\\ &= 1.34\end{aligned}$$

$$\begin{aligned}Relative\ TL\ (fold) &= 2^{-\Delta\Delta Cq(TL)}\\ &= 2^{-1.34}\\ &= 0.40\end{aligned}$$

$$\begin{aligned}Absolute\ TL\ (per\ diploid\ cell) &= Known\ Length \times 2^{-\Delta\Delta Cq(TL)}\\ &= (369.00 \pm 18.00\ kb) \times 0.40\\ &= 147.60 \pm 7.20\ kb\end{aligned}$$

$$\begin{aligned}Mean\ Absolute\ TL\ (per\ diploid\ cell) &= (147.60 \pm 7.20\ kb)\ /\ 92\\ &= 1.60 \pm 0.08\ kb\ per\ chromosome\ end.\end{aligned}$$

Thus, the average telomere length of the target genomic DNA sample is approximately 148 ± 7 kb per diploid cell, and 1.6 ± 0.1 kb per chromosome end.

Appendix F: Main effect of TL, mtDNAcn, perceived stress, and ACEs on cfPWV

Table S1. Linear regression main effect of TL (Model 1), mtDNAcn (Model 2), perceived stress, and ACEs on cfPWV (*n* = 185).

Model	Variable	*B*(SE)	*β*	95% CI		*p*
				LL	UL	
Model 1	Log(TL)	-0.356 (0.158)	-0.161	-0.668	-0.045	***0.025***
	Perceived Stress	0.023 (0.012)	0.150	0.001	0.047	***0.048***
	ACEs	0.092 (0.046)	0.150	0.002	0.183	***0.046***
Model 2	Log(mtDNAcn)	-0.438 (0.229)	-0.137	-0.890	0.013	*0.057*
	Perceived Stress	0.025 (0.012)	0.157	0.001	0.048	***0.039***
	ACEs	0.094 (0.046)	0.153	0.003	0.185	***0.043***

*R^2 of Model 1 = 0.090, p = 0.001; R^2 of Model 2 = 0.083, p = 0.001.

Appendix G: Interactions of TL and mtDNAcn with ACEs in predicting cfPWV

Table S2. Interaction effects of ACEs by TL (Model 1) and ACEs by mtDNAcn (Model 2) on cfPWV (*n* = 185).

Model	Variable	*B*(*SE*)	*β*	95% CI		*p*
				LL	UL	
Model 1	Age (Years)	0.104 (0.041)	0.170	0.022	0.186	***0.013***
	Sex†	0.120 (0.136)	0.067	-0.148	0.388	*0.377*
	Occ. Smoke‡	0.387 (0.246)	0.107	-0.098	0.872	*0.117*
	Reg. Smoke‡	0.506 (0.257)	0.146	-0.001	1.014	***0.050***
	BMI	0.037 (0.013)	0.221	0.011	0.062	***0.005***
	PA	-0.112 (0.060)	-0.131	-0.230	0.007	*0.064*
	MAP	0.013 (0.008)	0.126	-0.002	0.028	*0.097*
	HR	0.014 (0.006)	0.158	0.002	0.027	***0.027***
	Serum CRP	0.018 (0.129)	0.011	-0.237	0.272	*0.891*
	TL	-0.455 (0.240)	-0.205	-0.929	0.019	*0.060*
	ACEs	-0.030 (0.257)	-0.049	-0.538	0.478	*0.908*
	ACEs*TL	0.045 (0.097)	0.195	-0.146	0.235	*0.646*
Model 2	Age (Years)	0.101 (0.042)	0.165	0.019	0.183	***0.016***
	Sex†	0.111 (0.135)	0.062	-0.156	0.378	*0.414*
	Occ. Smoke‡	0.443 (0.245)	0.123	-0.041	0.927	*0.072*
	Reg. Smoke‡	0.576 (0.261)	0.166	0.061	1.091	***0.029***
	BMI	0.035 (0.013)	0.214	0.010	0.061	***0.007***
	PA	-0.105 (0.060)	-0.123	-0.224	0.014	*0.083*
	MAP	0.013 (0.008)	0.130	-0.002	0.029	*0.086*
	HR	0.016 (0.006)	0.172	0.003	0.028	***0.017***
	Serum CRP	0.017 (0.129)	0.011	-0.238	0.271	*0.898*
	mtDNAcn	-0.661 (0.350)	-0.206	-1.351	0.029	*0.061*
	ACEs	-0.115 (0.368)	-0.187	-0.841	0.611	*0.755*
	ACEs*mtDNAcn	0.074 (0.134)	0.333	-0.191	0.338	*0.583*

*Occ. Smoke indicates occasional smoking; Reg. Smoke, regular smoking; BMI, body mass index (kg/m^2); PA, physical activity (MET-minutes/week); MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); CRP, log-transformed C-reactive protein (ng/mL); TL, log-transformed absolute telomere length (kb per diploid cell); mtDNAcn, log-transformed mitochondrial DNA copy number (copies per diploid cell); ACEs*TL, ACEs by TL interaction term; ACEs*mtDNAcn, ACEs by mtDNAcn interaction term. *B* indicates the unstandardized regression coefficient; *SE*, standard error; *β*, standardized regression coefficient; *LL*, is the lower limit of the 95% confidence interval (95% CI); UL, upper limit; $p < 0.05$ indicates significance.

†Reference group for sex is male.

‡Reference group for smoking status is non-smokers.

§R^2 of Model 1 = 0.271, $p < 0.001$; R^2 of Model 2 = 0.268, $p < 0.001$.