Telomere Length and Vascular Phenotypes in a Population-Based Cohort of Children and Midlife Adults

Minh Thien Nguyen, BSc(Hons); Regan Vryer, BSc(Hons); Sarah Ranganathan, PhD; Kate Lyczett, PhD; Anneke Grobler, PhD; Terence Dwyer, MD; Markus Juonala, PhD; Richard Saffery, PhD; David Burgner, PhD; Melissa Wake, MD

Background—Telomere length has been inversely associated with cardiovascular disease in adulthood, but its relationship to preclinical cardiovascular phenotypes across the life course remains unclear. We investigated associations of telomere length with vascular structure and function in children and midlife adults.

Methods and Results—Population-based cross-sectional CheckPoint (Child Health CheckPoint) study of 11- to 12-year-old children and their parents, nested within the LSAC (Longitudinal Study of Australian Children). Telomere length (telomeric genomic DNA [T]/β-globin single-copy gene [S] [T/S ratio]) was measured by quantitative polymerase chain reaction from blood-derived genomic DNA. Vascular structure was assessed by carotid intima-media thickness, and vascular function was assessed by carotid-femoral pulse-wave velocity and carotid elasticity. Mean (SD) T/S ratio was 1.09 (0.55) in children (n=1206; 51% girls) and 0.81 (0.38) in adults (n=1343; 87% women). Linear regression models, adjusted for potential confounders, revealed no evidence of an association between T/S ratio and carotid intima-media thickness, carotid-femoral pulse-wave velocity, or carotid elasticity in children. In adults, longer telomeres were associated with greater carotid elasticity (0.14% per 10-mm Hg higher per unit of T/S ratio; 95% CI, 0.04%–0.2%; P=0.007), but not carotid intima-media thickness (−0.9 mm; 95% CI, −14 to 13 mm; P=0.9) or carotid-femoral pulse-wave velocity (−0.10 m/s; 95% CI, −0.3 to 0.07 m/s; P=0.2). In logistic regression analysis, telomere length did not predict poorer vascular measures at either age.

Conclusions—In midlife adults, but not children, there was some evidence that telomere length was associated with vascular elasticity but not thickness. Associations between telomere length and cardiovascular phenotypes may become more evident in later life, with advancing pathological changes. (J Am Heart Assoc. 2019;8:e012707. DOI: 10.1161/JAHA.119.012707.)

Key Words: aging • arterial stiffness • atherosclerosis • carotid intima-media thickness • CheckPoint (Child Health CheckPoint) study • LSAC (Longitudinal Study of Australian Children) • pulse-wave velocity

Given the global burden of cardiovascular disease (CVD), considerable efforts are under way to understand the underlying cause and to identify potential biomarkers predictive of risk.1 Cell senescence may play an important role in the progression of CVD, although the upstream drivers of CVD-associated cell death remain to be fully elucidated. One well-studied pathway to cellular senescence is mediated via telomere shortening. Telomeres are nucleoprotein structures that cap the ends of linear chromosomes. Their shortening represents a molecular mechanism of biological aging2 and occurs because of the general inability of cells to replicate the ends of linear DNA in the absence of telomerase. At a critical length, the DNA damage response drives cell senescence, resulting in inhibited tissue repair capacity and function.

Considerable evidence has linked shorter telomeres with greater risk of all-cause mortality,3 cancer,4 infectious disease,5 and CVD.6,7 Notably, a meta-analysis of 43 725 adults (mean...
Telomere Length and Vascular Phenotypes

Nguyen et al

DOI: 10.1161/JAHA.119.012707

Journal of the American Heart Association

that telomere length was not associated with carotid IMT. However, recent studies of healthy adults in late life showed shorter telomeres in clinical samples of older adults. Thicker carotid IMT has previously been associated with atherosclerosis and correlates with total atherosclerotic burden in adults. Faster carotid-femoral PWV and reduced carotid elasticity in adults is interesting; however, the strength of the association could equally be caused by chance and, hence, requires replication.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Participants

The LSAC (Longitudinal Study of Australian Children) recruited a nationally representative birth cohort in 2004 (n=5107). Participants have been followed up at 7 biennial waves spanning birth to 13 years. The CheckPoint (Child Health CheckPoint) study was an additional physical health and biomarker wave for the birth cohort, nested between LSAC’s sixth and seventh waves. During the LSAC wave 6 assessment in 2014, interviewers obtained written consent from 3513 families (93% of the 3764 seen) to be contacted to participate in the upcoming CheckPoint study. Ultimately, 1874 adults-child pairs (50%) took part. Most nonparticipation (60%) was caused by inability to attend or to reschedule a visit during the short period CheckPoint study was in each location. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Data collection ran from February 2015 to March 2016. The CheckPoint study Assessment Center operated sequentially across Australia in major cities (3.5 hours, main center) and regional centers (2.5 hours, minicenter), with home visits offered to those unable to attend a center. Children and their attending parent rotated through a series of 15-minute stations, in which different aspects of health were assessed. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Noninvasive measures of vascular phenotypes enable assessment of cardiovascular structure and function in the large arteries. Ultrasound and tonometry of the carotid artery can capture carotid intima-media thickness (IMT), carotid-femoral pulse-wave velocity (PWV), and carotid elasticity. Carotid IMT is a validated structural marker of subclinical atherosclerosis and correlates with total atherosclerotic burden in adults. Thicker carotid-femoral PWV and reduced carotid elasticity are functional measures of arterial stiffness linked to higher risk of cardiovascular events. Thicker carotid IMT has previously been associated with shorter telomeres in clinical samples of older adults. However, recent studies of healthy adults in late life showed that telomere length was not associated with carotid IMT or with carotid atherosclerosis. Functional measures have also been linked with shortened telomeres in later-life adults.

Although telomere length may be a potential mechanism underlying CVD across the life course, studies are conflicting and little is known of the relationship in younger adults or children. It may be that telomere length is associated with vascular function earlier than structural phenotypes. Thus, exploring the relationship of telomere length with functional and structural phenotypes across the life course could enhance our understanding of the role of telomere length-driven cell senescence in the pathogenesis of CVD. We aimed to assess the associations of telomere length with measures of vascular structure and function in a population-based cross-sectional study of children and their parents.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Participants

The LSAC (Longitudinal Study of Australian Children) recruited a nationally representative birth cohort in 2004 (n=5107). Participants have been followed up at 7 biennial waves spanning birth to 13 years. The CheckPoint (Child Health CheckPoint) study was an additional physical health and biomarker wave for the birth cohort, nested between LSAC’s sixth and seventh waves. During the LSAC wave 6 assessment in 2014, interviewers obtained written consent from 3513 families (93% of the 3764 seen) to be contacted to participate in the upcoming CheckPoint study. Ultimately, 1874 adults-child pairs (50%) took part. Most nonparticipation (60%) was caused by inability to attend or to reschedule a visit during the short period CheckPoint study was in each location. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Data collection ran from February 2015 to March 2016. The CheckPoint study Assessment Center operated sequentially across Australia in major cities (3.5 hours, main center) and regional centers (2.5 hours, minicenter), with home visits offered to those unable to attend a center. Children and their attending parent rotated through a series of 15-minute stations, in which different aspects of health were assessed. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Noninvasive measures of vascular phenotypes enable assessment of cardiovascular structure and function in the large arteries. Ultrasound and tonometry of the carotid artery can capture carotid intima-media thickness (IMT), carotid-femoral pulse-wave velocity (PWV), and carotid elasticity. Carotid IMT is a validated structural marker of subclinical atherosclerosis and correlates with total atherosclerotic burden in adults. Thicker carotid-femoral PWV and reduced carotid elasticity are functional measures of arterial stiffness linked to higher risk of cardiovascular events. Thicker carotid IMT has previously been associated with shorter telomeres in clinical samples of older adults. However, recent studies of healthy adults in late life showed that telomere length was not associated with carotid IMT or with carotid atherosclerosis. Functional measures have also been linked with shortened telomeres in later-life adults.

Although telomere length may be a potential mechanism underlying CVD across the life course, studies are conflicting and little is known of the relationship in younger adults or children. It may be that telomere length is associated with vascular function earlier than structural phenotypes. Thus, exploring the relationship of telomere length with functional and structural phenotypes across the life course could enhance our understanding of the role of telomere length-driven cell senescence in the pathogenesis of CVD. We aimed to assess the associations of telomere length with measures of vascular structure and function in a population-based cross-sectional study of children and their parents.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Participants

The LSAC (Longitudinal Study of Australian Children) recruited a nationally representative birth cohort in 2004 (n=5107). Participants have been followed up at 7 biennial waves spanning birth to 13 years. The CheckPoint (Child Health CheckPoint) study was an additional physical health and biomarker wave for the birth cohort, nested between LSAC’s sixth and seventh waves. During the LSAC wave 6 assessment in 2014, interviewers obtained written consent from 3513 families (93% of the 3764 seen) to be contacted to participate in the upcoming CheckPoint study. Ultimately, 1874 adults-child pairs (50%) took part. Most nonparticipation (60%) was caused by inability to attend or to reschedule a visit during the short period CheckPoint study was in each location. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Data collection ran from February 2015 to March 2016. The CheckPoint study Assessment Center operated sequentially across Australia in major cities (3.5 hours, main center) and regional centers (2.5 hours, minicenter), with home visits offered to those unable to attend a center. Children and their attending parent rotated through a series of 15-minute stations, in which different aspects of health were assessed. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Noninvasive measures of vascular phenotypes enable assessment of cardiovascular structure and function in the large arteries. Ultrasound and tonometry of the carotid artery can capture carotid intima-media thickness (IMT), carotid-femoral pulse-wave velocity (PWV), and carotid elasticity. Carotid IMT is a validated structural marker of subclinical atherosclerosis and correlates with total atherosclerotic burden in adults. Thicker carotid-femoral PWV and reduced carotid elasticity are functional measures of arterial stiffness linked to higher risk of cardiovascular events. Thicker carotid IMT has previously been associated with shorter telomeres in clinical samples of older adults. However, recent studies of healthy adults in late life showed that telomere length was not associated with carotid IMT or with carotid atherosclerosis. Functional measures have also been linked with shortened telomeres in later-life adults.

Although telomere length may be a potential mechanism underlying CVD across the life course, studies are conflicting and little is known of the relationship in younger adults or children. It may be that telomere length is associated with vascular function earlier than structural phenotypes. Thus, exploring the relationship of telomere length with functional and structural phenotypes across the life course could enhance our understanding of the role of telomere length-driven cell senescence in the pathogenesis of CVD. We aimed to assess the associations of telomere length with measures of vascular structure and function in a population-based cross-sectional study of children and their parents.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Participants

The LSAC (Longitudinal Study of Australian Children) recruited a nationally representative birth cohort in 2004 (n=5107). Participants have been followed up at 7 biennial waves spanning birth to 13 years. The CheckPoint (Child Health CheckPoint) study was an additional physical health and biomarker wave for the birth cohort, nested between LSAC’s sixth and seventh waves. During the LSAC wave 6 assessment in 2014, interviewers obtained written consent from 3513 families (93% of the 3764 seen) to be contacted to participate in the upcoming CheckPoint study. Ultimately, 1874 adults-child pairs (50%) took part. Most nonparticipation (60%) was caused by inability to attend or to reschedule a visit during the short period CheckPoint study was in each location. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Data collection ran from February 2015 to March 2016. The CheckPoint study Assessment Center operated sequentially across Australia in major cities (3.5 hours, main center) and regional centers (2.5 hours, minicenter), with home visits offered to those unable to attend a center. Children and their attending parent rotated through a series of 15-minute stations, in which different aspects of health were assessed. Details of the LSAC and CheckPoint study design and recruitment have been previously described, and the Figure shows the flow through the study.

Noninvasive measures of vascular phenotypes enable assessment of cardiovascular structure and function in the large arteries. Ultrasound and tonometry of the carotid artery can capture carotid intima-media thickness (IMT), carotid-femoral pulse-wave velocity (PWV), and carotid elasticity. Carotid IMT is a validated structural marker of subclinical atherosclerosis and correlates with total atherosclerotic burden in adults. Thicker carotid-femoral PWV and reduced carotid elasticity are functional measures of arterial stiffness linked to higher risk of cardiovascular events. Thicker carotid IMT has previously been associated with shorter telomeres in clinical samples of older adults. However, recent studies of healthy adults in late life showed that telomere length was not associated with carotid IMT or with carotid atherosclerosis. Functional measures have also been linked with shortened telomeres in later-life adults.

Although telomere length may be a potential mechanism underlying CVD across the life course, studies are conflicting and little is known of the relationship in younger adults or children. It may be that telomere length is associated with vascular function earlier than structural phenotypes. Thus, exploring the relationship of telomere length with functional and structural phenotypes across the life course could enhance our understanding of the role of telomere length-driven cell senescence in the pathogenesis of CVD. We aimed to assess the associations of telomere length with measures of vascular structure and function in a population-based cross-sectional study of children and their parents.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.
cryopreserved until depletion. The logistics and specified requirements of this large, national, government-owned population-based cohort precluded any participants attending repeated sessions. However, all values for all participants in both age groups are based on repeated measurements within the same session, as are the interobserver and intraobserver ratings for the observer-dependent measurements (carotid IMT and waveform quality ratings). Previous studies have confirmed similar repeatability between children and adults, and between sessions separated by hours to weeks. The study was approved by the Royal Children’s Hospital Melbourne Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26). The attending parents provided written informed consent for themselves and their child before participation, and the child provided assent.

### DNA Isolation and Telomere Length Measurement

Genomic DNA was isolated from available blood using the QIAamp 96 DNA Blood Kit (Qiagen, Venlo, the Netherlands). Purity and integrity was confirmed using the NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Middleton, WI), the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA), and gel electrophoresis. Telomere length was measured with the quantitative real-time polymerase chain reaction method, originally described by Cawthon. This method measures the amount of telomeric genomic DNA (T)/β-globin single-copy gene (S) (T/S ratio) for each participant. The mean intra-assay variability between “T” and “S” quadruplicates used in the calculation of the T/S ratio was 1.7% (SD, 0.3%; range, 0.9%–2.6%). The interassay variability between plates was 1.7% (SD, 1.4%; range, 0.3%–6.2%). Further details on the telomere length calculation (Table S1), plate conditions, and constituents are described in Data S1 and in the Standard Operating Procedure on the CheckPoint study’s website.

### Carotid IMT and Carotid Elasticity

Carotid IMT and elasticity were measured using standardized protocols via ultrasound (Vivid i BT06 with 10-MHz linear array probe; GE Healthcare, Little Chalfont, UK). Ultrasounds were performed in supine position with head turned 45° to the...
left. The ultrasound probe was applied to the right side of the neck at \(\approx 45^\circ\) to the midline with concurrent 3-lead ECG trace. The duration of the captured real-time B-mode ultrasound cine loops was 10 cardiac cycles.

All images were reviewed for quality to select optimal loops comprising a clear near and far wall intima-media, clear lumen, straight vessel, presence of the carotid bulb, and an ECG trace. The best-quality 5 to 7 cardiac cycle section of the loops was trimmed and extracted. These loops were further processed using Carotid Analyzer (Medical Imaging Applications, Coralville, IA). Raters calibrated the images using ultrasound image markers. Maximum carotid IMT values were used in analyses, which refer to the 3 to 5 frame average of the thickest point of carotid IMT measurement over the highest quality 5- to 10-mm section from the carotid bulb. After algorithmic detection of the intima-media interface over the entire cine-loop, frames were manually adjusted as needed or rejected if the intima-media interface was unclear or blurred. The intraobserver variability for the maximum carotid IMT values was 4.9% (95% CI, 4.6%–5.2%), and the interobserver variability was 6.2% (95% CI, 5.2%–7.2%). Further details are described elsewhere.\(^{25,34}\)

Carotid elasticity was calculated from carotid artery images and expressed as a percentage change in intima-intima lumen diameter (measured from ultrasound) per change in pulse pressure (measured from SphygmoCor), as previously described (Table S1).\(^{13}\) Intima-intima lumen diameter measurement was automated using Carotid Analyzer and, thus, was rater independent; it was calculated by measuring the average intima-intima distance (subtracting near and far wall IMT measurements) on each of the 3 to 5 still frames used to calculate maximum carotid IMT. Intraobserver and interobserver variability values for maximum lumen diameter were 1.3% (95% CI, 1.2%–1.4%) and 1.6% (95% CI, 1.4%–1.9%), respectively; and values for minimum lumen diameter were 1.2% (95% CI, 1.1%–1.3%) and 1.5% (95% CI, 1.2%–1.7%), respectively. Further details are in Data S1 and prior publications.\(^{25,34}\)

**Carotid-Femoral PWV and Blood Pressure**

Carotid-femoral PWV and blood pressure were measured using tonometry via SphygmoCor XCEL (AtCor Medical, West Ryde, Australia), as previously described.\(^{14}\) Carotid-femoral PWV was determined by detecting waveforms simultaneously by a hand-held tonometer at the carotid pulse and a cuff placed on the proximal thigh, over a 10-second period. This was completed 3 times in the supine position. The distance traveled by waveforms was measured with a tape measure from the carotid pulse to the suprasternal notch, from the suprasternal notch to the right femoral pulse, and from the femoral pulse to the top of the thigh cuff. The mean of at least 2 valid carotid-femoral PWV measurements (in m/s) was used in analyses. Systolic and diastolic blood pressure values were recorded at the brachial artery, 3 times, 1 minute apart, with either a 23- to 33-cm or a 31- to 40-cm cuff, depending on arm size. Systolic and diastolic blood pressure values were included if participants had at least 2 valid measurements. Further details of these measures are described elsewhere.\(^{25,35}\)

At the time of assessment, waveforms were assessed using the in-built quality control software in the SphygmoCor XCEL. After study completion, waveforms were further reviewed by 2 trained analysts and were excluded if they did not meet quality control standards. To assess interrater reliability, 112 individually recorded waves from a random sample of 40 participants (20 children and 20 adults) from the CheckPoint study database were presented blindly to 2 analysts for review. Pulse wave quality ratings given by each analyst (poor, adequate, or good) were compared by calculating the proportion of positive agreement between analysts. Most sample waveforms were of good quality, and none were of poor quality. The overall correlation between analysts was high (\(r=0.99\)). Automated measurement of carotid-femoral PWV by SphygmoCor is approved by the US Food and Drug Administration, is extensively standardized across large general populations,\(^{22,36,37}\) and is validated against other well-validated tonometry-based methods\(^{38,39}\) as per the ARTERY (Association for Research into Arterial Structure and Physiology) PWV validation guidelines.\(^{40}\) Published values for interobserver and intraobserver carotid-femoral PWV variability are 0.30 m/s (SD, 1.25 m/s) and 0.07 m/s (SD, 1.17 m/s), respectively.\(^{41}\) Further details are in Data S1 and are described elsewhere.\(^{25,35}\)

**Potential Confounders**

Several variables were considered a priori as potential confounders, including body mass index (BMI; kg/m\(^2\)),\(^{42}\) socioeconomic status,\(^{43}\) and smoking.\(^{44}\) Each of these variables has consistently been associated with telomere length, as well as being commonly known risk factors for cardiovascular health. BMI was calculated from height (standard portable stadiometer; IP0955; Invicta, Leicester, UK) and weight (InBody230 bioelectrical impedance analysis scale; Biospace Co Ltd Seoul, South Korea). For children, an age- and sex-adjusted BMI \(z\) score was calculated using the US Centers for Disease Control and Prevention growth reference charts.\(^{45}\) As a measure of neighborhood socioeconomic status, we used the Socio-Economic Indexes for Areas Index of Relative Disadvantage score, which is based on the postcode of domicile of the participating family. This is a standardized score by geographic area, compiled from 2011 Australian Census data to numerically summarize the social

DOI: 10.1161/JAHA.119.012707
Telomere Length and Vascular Phenotypes

Nguyen et al

and economic conditions of Australian neighborhoods (national mean of 1000 and SD of 100, where higher values represent less disadvantage). Parental current smoking behavior and cigarettes smoked per day were self-reported at LSAC wave 6. Preexisting conditions were self-reported (yes/no) by questionnaire at the CheckPoint study assessment, including diabetes mellitus status, heart conditions, hypertension medication, and pacemakers. Further details of these sample measures are described elsewhere.25

Other Covariates

Other covariates traditionally associated with cardiovascular outcomes were measured using the Nightingale nuclear magnetic resonance metabolomics platform (Helsinki, Finland) from blood serum. These included lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), glucose, and the inflammation marker of glycoprotein acetyls. Further details of this platform and method are extensively described elsewhere.46

Statistical Analysis

Stata 14.2 (StataCorp, College Station, TX) was used for all analyses. Continuous variables are presented as means and SD, whereas dichotomous variables are presented as percentages.

Associations of telomere length with vascular measures were examined by linear and logistic regression models, in children and adults separately. Assumptions for linear regression were examined using histograms and scatterplots, and they showed no discernible outliers and minimal right skewing for child’s and adult’s telomere length. For aim 1, linear regression models fitted continuous telomere length as the independent variable and continuous data on vascular measures as dependent variables in separate models. For aim 2, logistic regression models fitted continuous telomere length as the independent variable and elevated carotid IMT, carotid-femoral PWV, and reduced carotid elasticity as the dependent variables in separate models. Elevated carotid IMT and elevated carotid-femoral PWV were defined internally as >75th percentile for the sample. Reduced carotid elasticity was defined internally as <25th percentile.

For both aims, model 1 included adjustments for the potential lifelong confounders of sex and age, as well as for sample type (ie, whole blood or blood clot) to account for any effects of the early change in the sample collection protocol from blood clots to whole blood. Adjusted analyses included the covariates as independent variables. Model 2 additionally included adjustments for the additional potential confounders of BMI and Socio-Economic Indexes for Areas Index of Relative Disadvantage score, plus smoking status, for adults. In model 3, we further adjusted for systolic blood pressure, lipids, glucose, and glycoprotein acetyls. Finally, we conducted sensitivity analysis that did the following: (1) further adjusted for preexisting conditions, (2) adjusted for mean arterial pressure or diastolic blood pressure in place of systolic blood pressure, and (3) stratified by, rather than adjusted for, sample type. Sensitivity analyses showed similar results to those presented below (data not shown; available on request).

Results

A total of 1874 parent-child dyads participated in the CheckPoint study (Figure). As venous blood was not collected at home visits (n=364 dyads), these participants were not included in the current analysis. Venous blood (whole blood or blood clot) was available for 1216 children and 1350 adults. Telomere length data were generated for 1206 children (99.2%) and 1343 adults (99.5%).

Sample Characteristics

Participant characteristics are displayed in Table 1. Children and adults were, on average, aged 11 years (SD, 0.5 years) and 44 years (SD, 5.1 years), respectively. Adults were predominantly mothers (n=1168; 87%), whereas there were approximately equal numbers of boys and girls. Participants came from slightly less disadvantaged and more homogeneous areas compared with the national average (mean Socio-Economic Indexes for Areas Index of Relative Disadvantage score, 1026 [SD, 61] versus 1000 [SD, 100]). Child and adult BMI scores were similar to those of the current Australian population, as recorded by the Australian Bureau of Statistics, with ≈1 in 4 children and 2 in 3 adults overweight or obese.47 Compared with the general Australian population rates for similar age ranges, adults’ self-report of diabetes mellitus (2.4% versus 4%) and being a current smoker (8% versus 16%) were lower,47 but CVD (2.2% versus 2.5%) was comparable.47

Children had longer telomeres (higher T/S ratio, 1.09; SD, 0.56) than adults (0.81; SD, 0.38).33 Adults, on average, had thicker carotid IMT than children (663 [SD, 97] μm versus 580 [SD, 46] μm) and stiffer vessels, as shown in faster carotid-femoral PWV (7.0 [SD, 1.1] m/s versus 4.4 [SD, 0.5] m/s) and lower carotid elasticity (2.4% [SD, 0.6%] versus 4.8% [SD, 0.9%] per 10-mm Hg). Further details of the distributions of vascular phenotypes are in Figure S1 and were previously described.34,35 Adults with self-reported diabetes mellitus and hypertension medication use had thicker carotid IMT, faster carotid-femoral PWV, and lower carotid elasticity (Table S2). Vascular phenotypes were similar in current
Telomere Length and Vascular Phenotypes

Nguyen et al

Table 1. Summary Characteristics of Children and Adults

| Participant Characteristic | Children | Adults |
|---------------------------|----------|-------|
| Total No.                 | 1206     | 1343  |
| Age, y                    | 11 (0.5) | 44 (5.1) |
| Female sex, %             | 51       | 87    |
| Height, cm                | 154 (8)  | 167 (8) |
| Body mass index, kg/m²    | 19 (3)   | 28 (6) |
| Body mass index z score   | 0.3 (1)  | ...   |
| Disadvantage score        | 1026 (62) | 1026 (61) |
| Current smoking, %        | ...      | 8.2   |
| Cigarettes smoked per day | ...      | 2.6 (0.14) |
| Preexisting conditions, % |          |       |
| Diabetes mellitus         | 0.1      | 2.4   |
| Heart condition           | ...      | 2.2   |
| Hypertension medication   | ...      | 5.1   |
| Pacemakers                | ...      | 0.1   |
| Systolic blood pressure, mm Hg | 108 (8) | 120 (13) |
| Diastolic blood pressure, mm Hg | 63 (6) | 74 (9) |
| Lumen diameter, mm        | 5.9 (0.5) | 5.8 (0.6) |
| HDL cholesterol, mg/dL    | 54 (10)  | 56 (14) |
| LDL cholesterol, mg/dL    | 25 (6)   | 30 (8) |
| Total cholesterol, mg/dL  | 73 (12)  | 86 (16) |
| Triglycerides, mg/dL      | 105 (49) | 132 (75) |
| Glucose, mg/dL            | 89 (14)  | 88 (19) |
| Glycoprotein acetylation, mmol/L | 0.99 (0.13) | 1.04 (0.17) |
| Telomere length (T/S ratio) | 1.09 (0.55) | 0.81 (0.38) |
| Vascular outcomes         |          |       |
| Carotid intima-media thickness, μm | 580 (46) | 663 (97) |
| Carotid-femoral pulse wave velocity, m/s | 4.4 (0.5) | 7.0 (1.1) |
| Carotid elasticity, % per 10-mm Hg | 4.8 (0.9) | 2.4 (0.6) |

Data are presented as mean (SD) or percentage. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; T/S ratio, telomeric genomic DNA (T)/interleukin-1β (IL-1β) single-copy gene (S).

Discussion

Main Findings

In the present study, we hypothesized that telomere attrition is an upstream event of cardiometabolic disease and, as such, evidence for shortened telomeres will be present in mid-aged adults and children in association with preclinical phenotypes of poorer cardiovascular health. Furthermore, given the progressive nature of the proposed association, we predicted that the strength of association between telomere length and phenotypes will be stronger in adults relative to their children. We tested this in a population-based cohort of children and mid-life adults and found no convincing evidence of associations between shorter telomeres and adverse vascular phenotypes at either age, other than a small association between longer telomere length and increased carotid elasticity in adults.

Strengths and Limitations

Strengths of our study include the objective examination of carotid IMT, carotid-femoral PWV, and carotid elasticity with high reliability and the T/S measurements showing low replicate variation. We are confident in interpreting the child and adult findings within the same study because they were assessed at the same time point with the same protocols, equipment, and staff. For telomere length, we used a quantitative real-time polymerase chain reaction method that correlates strongly with the gold standard terminal restriction fragment analysis. However, although well suited for large epidemiological studies, this method does not quantify absolute, chromosome-specific, or vascular tissue-specific telomere length. Also, the capacity of telomere length derived...
from blood as a surrogate for telomere length within the vasculature itself is uncertain; however, there is evidence that telomere length is highly conserved between tissues.48,49 Differential uptake in LSAC and attrition in CheckPoint study led to our sample including few fathers and few less disadvantaged families than in the general Australian population. Thus, if such children and adults had both shorter telomeres and poorer vascular outcomes, this could have altered estimates and/or their precision. Finally, the cross-sectional design precluded conclusions about temporal associations.

**Interpretation in Light of Other Studies**

**Telomere length, vascular structure, and atherosclerosis**

We found little evidence of an association between telomere length and carotid IMT. This is consistent with several prior reports showing no or only marginal associations.17,19,20,50–52 For example, in healthy adults, telomere length was not independently associated with carotid IMT19 (n=2509; age range, 35–55 years) and atherosclerosis in the carotid arteries (n=1459; age range, 40–54 years).20 Together, these studies suggest that associations between telomere length and vascular structure appear primarily later in adulthood. Moreover, there is evidence that shortened telomere length is more reliably associated with patients with CVD. For example, shorter telomere length has been observed in aortic tissues with atherosclerotic lesions, compared with tissues without atherosclerotic lesions;8 and in another study, telomere length was associated with advanced-state, but not with early-stage, atherosclerosis.51 Telomere shortening might play a role late in the pathogenesis of CVD, in late adulthood when the effects of cumulative burden over the life course begin to manifest. This hypothesis would be consistent with current knowledge, in which the cellular senescence induced by the aging process is known to trigger the progressive deterioration of vascular functionality with age.53

Our findings also align with evidence that shortened telomere length is associated with vascular phenotypes in those with greater CVD risk6 (eg, in elderly populations; mean age, 74.2 years; SD, 5.2 years),17 diabetic subjects,9,54 and obese men.50 Interestingly, a recent meta-analysis of 5566 patients with coronary artery disease found an overall higher risk for coronary artery disease in older subjects (≥70 years), compared with younger subjects (<70 years; relative risk, 1.9 versus 1.5), with respect to individuals in the shortest telomere tertile compared with those in the longest telomere tertile compared with those in the shortest telomere tertile versus 1.5), with respect to individuals in the shortest telomere tertile compared with those in the longest telomere tertile.

**Telomere length, vascular function, and arterial stiffness**

There was little evidence of an association between telomere length and carotid-femoral PWV in children or adults. Several

| Outcome | Children | Adults |
|---------|----------|--------|
|         | Coefficient (95% CI) | R² | P Value | No. | Coefficient (95% CI) | R² | P Value |
| Model 1* |          |        |        |       |                  |     |         |
| Carotid IMT, μm | 1193 | 4.11 (−1.21 to 9.4) | 0.04 | 0.13 | 1203 | −0.85 (−14.31 to 12.61) | 0.16 | 0.90 |
| Carotid-femoral PWV, m/s | 1167 | −0.01 (−0.07 to 0.06) | 0.06 | 0.86 | 1110 | −0.10 (−0.27 to 0.07) | 0.10 | 0.23 |
| Carotid elasticity, % per 10-mm Hg | 1083 | −0.04 (−0.15 to 0.06) | 0.01 | 0.42 | 1040 | 0.14 (0.04 to 0.24) | 0.12 | 0.007 |
| Model 2† |          |        |        |       |                  |     |         |
| Carotid IMT, μm | 1192 | 4.67 (−0.64 to 9.99) | 0.05 | 0.09 | 1198 | 0.46 (−12.71 to 13.64) | 0.20 | 0.95 |
| Carotid-femoral PWV, m/s | 1166 | 0.01 (−0.05 to 0.07) | 0.13 | 0.68 | 1106 | −0.12 (−0.28 to 0.04) | 0.24 | 0.13 |
| Carotid elasticity, % per 10-mm Hg | 1082 | −0.06 (−0.17 to 0.05) | 0.04 | 0.26 | 1035 | 0.13 (0.04 to 0.22) | 0.26 | 0.006 |
| Model 3‡ |          |        |        |       |                  |     |         |
| Carotid IMT, μm | 1082 | 4.89 (−0.60 to 10.37) | 0.07 | 0.08 | 1082 | 1.17 (−12.60 to 14.94) | 0.25 | 0.87 |
| Carotid-femoral PWV, m/s | 1070 | 0.02 (−0.04 to 0.08) | 0.16 | 0.57 | 1031 | −0.09 (−0.23 to 0.06) | 0.40 | 0.24 |
| Carotid elasticity, % per 10-mm Hg | 1037 | −0.09 (−0.19 to 0.02) | 0.15 | 0.11 | 1003 | 0.09 (0.003 to 0.18) | 0.36 | 0.04 |

IMT indicates intima-media thickness; PWV, pulse-wave velocity; R², value for the linear regression model.

*Model 1 was adjusted for sex, age, and sample type.
†Model 2 was additionally adjusted for body mass index, Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage score, plus smoking status for adults.
‡Model 3 was additionally adjusted for brachial systolic blood pressure, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and glycoprotein acetylation.
### Table 3. Odds Ratio for Elevated Carotid IMT, Elevated Carotid-Femoral PWV, and Reduced Carotid Elasticity for 1 Unit Higher T/S Ratio, in Children and Adults

| Outcome                        | Children          | Adults            | P Value | Children          | Adults            | P Value |
|--------------------------------|-------------------|-------------------|---------|-------------------|-------------------|---------|
|                                | No.   | Odds Ratio (95% CI) |        | No.   | Odds Ratio (95% CI) |        |
| Model 1*                       |       |                    |        |       |                    |        |
| Elevated carotid IMT           | 1193  | 1.23 (0.94–1.61)    | 0.13    | 1203  | 0.94 (0.65–1.37)    | 0.77    |
| Elevated carotid-femoral PWV   | 1167  | 0.82 (0.60–1.13)    | 0.23    | 1110  | 0.88 (0.59–1.30)    | 0.56    |
| Reduced carotid elasticity     | 1083  | 0.99 (0.73–1.32)    | 0.92    | 1040  | 0.84 (0.55–1.30)    | 0.44    |
| Model 2†                       |       |                    |        |       |                    |        |
| Elevated carotid IMT           | 1192  | 1.27 (0.97–1.66)    | 0.08    | 1198  | 0.98 (0.67–1.44)    | 0.92    |
| Elevated carotid-femoral PWV   | 1166  | 0.88 (0.63–1.23)    | 0.47    | 1106  | 0.83 (0.54–1.28)    | 0.40    |
| Reduced carotid elasticity     | 1082  | 1.02 (0.76–1.38)    | 0.91    | 1035  | 0.83 (0.52–1.32)    | 0.42    |
| Model 3‡                       |       |                    |        |       |                    |        |
| Elevated carotid IMT           | 1082  | 1.26 (0.95–1.68)    | 0.11    | 1082  | 1.03 (0.66–1.61)    | 0.89    |
| Elevated carotid-femoral PWV   | 1070  | 0.97 (0.68–1.38)    | 0.86    | 1031  | 0.73 (0.43–1.24)    | 0.25    |
| Reduced carotid elasticity     | 1037  | 1.16 (0.84–1.58)    | 0.37    | 1033  | 1.03 (0.61–1.74)    | 0.92    |

Elevated carotid IMT defined as >75th percentile, elevated carotid-femoral PWV defined as >75th percentile, and reduced carotid elasticity defined as <25th percentile. IMT indicates intima-media thickness; PWV, pulse-wave velocity; T/S ratio, telomeric genomic DNA (T)/β-globin single-copy gene (S).

*Model 1 was adjusted for sex, age, and sample type.
†Model 2 was additionally adjusted for body mass index, Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage score, plus smoking status for adults.
‡Model 3 was additionally adjusted for brachial systolic blood pressure, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and glycoprotein acetylation.

Recent studies have reported an association between shorter telomeres and faster carotid-femoral PWV, but most have been in older adults (>50 years), were limited by modest sample sizes (range, 49–303 individuals), and were predominately in men. For example, Benetos et al showed that shorter telomere length was significantly associated with faster carotid-femoral PWV in men (n=120; mean age, 55 years; r=-0.14), but not in women (n=73; mean age, 56 years; r=-0.05). Similar results were also found in a study of healthy Chinese men (n=112; mean age, 56 years). We cannot tell whether our lack of association between telomere length and carotid-femoral PWV reflects our preponderance of women or the younger age in our sample. Interestingly, previous findings suggest that age significantly modifies the relationship between telomere length and carotid-femoral PWV reflects our preponderance of women or the younger age in our sample. Interestingly, previous findings suggest that age significantly modifies the relationship between telomere length and carotid-femoral PWV reflects our preponderance of women or the younger age in our sample. Interestingly, previous findings suggest that age significantly modifies the relationship between telomere length and carotid-femoral PWV reflects our preponderance of women or the younger age in our sample.

In summary, we report a novel, albeit small, association between telomere length and carotid elasticity in adults, which remained after adjustment for a priori specified confounders. Although speculative at this point, this suggests a possibility that cellular senescence might contribute to vascular function, through the elasticity of the vasculature. One hypothesis might be that the secretory phenotype of senescent vascular cells might promote vascular degeneration through the destabilization of extracellular matrix, elastin, collagen, and smooth muscle, thus modifying the elasticity of the carotid artery. However, we suspect that it would also have effects on overall arterial stiffness and, thus, affect carotid-femoral PWV, which we did not find. This discordance warrants further investigation. We note that although vascular structure and function are considered to be related, they usually measure different properties of the arterial tree, both likely affected by risk factors in a nonuniform manner. It has been documented that functional changes precede the development of structural alterations in the vasculature.

Clinical Implications, Unanswered Questions, and Future Direction

Our findings, if replicated in other settings, question the clinical utility of measuring telomere length in healthy relatively young populations. The magnitude of the cross-sectional association between telomere length and carotid elasticity observed among adults was small. In addition, the European Society of Hypertension and the European Society of Cardiology noted that a carotid IMT of >900 μm and a carotid-femoral PWV of >12 m/s are associated with increased CVD risk, thresholds not reached by our relatively healthy cohort of children and adults. This suggests that the magnitude of the associated changes is unlikely to represent clinically significant differences on CVD risk, especially in largely healthy populations of children or mid-life adults. However, telomere length might be of greater clinical...
relevance at an advanced age or in association with more pronounced vascular pathological features. Moreover, it is possible that such associations could be important longitudinally, such that the rate of telomere attrition, rather than cross-sectional telomere length per se, may be a more clinically important cardiovascular risk marker. Indeed, in a recent British longitudinal study (baseline n=2611; mean age, 53 years; follow-up age range, 60–64 years), an association was observed between the rate of telomere attrition and thicker carotid IMT at 60 to 64 years, despite limited cross-sectional associations.18 Future studies should consider longitudinal evaluation with repeated telomere sampling to assess whether telomere attrition over time is associated with vascular phenotypes and cardiovascular events. We cannot exclude the possibility that our findings may have arisen by chance, and might be explained by factors beyond the scope of this study, including genetic variation and other unmeasured environmental exposures. The ability of carotid ultrasounds and tonometry to effectively measure meaningful changes in vascular structure and function in healthy individuals is also uncertain. In particular, the predictive value of carotid IMT measurement in children aged 11 to 12 years without CVD risk remains unclear.57

Conclusion
In a healthy cohort of children and mid-life adults, we report limited evidence that cellular senescence, as measured by telomere length, is associated with vascular structure or function in children or adults. The novel small association between longer telomere length and higher carotid elasticity in adults is interesting; supporting our hypothesis that telomere length may be on the mechanistic pathway toward impaired vascular function; however, the strength of the association could equally be caused by chance and, hence, requires replication. Although telomere length may be important for individuals at intermediate to high CVD risk or those at advanced disease stages, as previously reported, cross-sectional telomere length assessment appears to have limited clinical value for CVD risk in healthy populations, particularly at younger ages. The interaction between telomere dynamics and vascular phenotypes across the life course is multifaceted, with contributions from genetic variation and environmental exposures. Further longitudinal investigations, with repeated telomere and vascular phenotypic sampling, are warranted.

Acknowledgments
This article uses data from the LSAC (Longitudinal Study of Australian Children). The study is conducted in partnership between the Department of Social Services, the Australian Institute of Family Studies, and the Australian Bureau of Statistics. The findings and views reported are those of the authors. We thank the LSAC and CheckPoint (Child Health CheckPoint) study participants, staff, and students for their contributions.

Sources of Funding
This work was supported by the National Health and Medical Research Council (NHMRC) of Australia (Project Grants 1041352 and 1109355), The Royal Children’s Hospital Foundation (2014-241), the Murdoch Children’s Research Institute (MCRI), The University of Melbourne, the National Heart Foundation of Australia (100660), and Financial Markets Foundation for Children (2014-055 and 2016-310). The MCRI administered the grants for the study and provided infrastructural support (information technology and biospecimen management), but played no role in the conduct or analysis of the trial. Research at the MCRI is supported by the Victorian Government’s Operational Infrastructure Support Program. Nguyen was supported by an NHMRC Postgraduate Scholarship (1115167). Saffery was supported by an NHMRC Senior Research Fellowship (1045161). Ranganathan was supported by an MCRI Clinician Scientist Award. Lyczek was supported by an NHMRC Early Career Fellowship (109124) and a National Heart Foundation Postdoctoral Fellowship (101239). Grobler and Dwyer reported no sources of funding. Juonala was supported by the Federal Research Grant of Finland to Turku University Hospital and the Juho Vainio Foundation. Burgner was supported by an NHMRC Fellowship (1064629) and an Honorary Future Leader Fellowship of the National Heart Foundation of Australia (100369). Wake was supported by an NHMRC Senior Research Fellowship (1046518) and Cure Kids New Zealand. The funding bodies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclosures
None.

References
1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Iasigi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER III, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2016 update: a report from the American Heart Association. Circulation. 2016;133:e38–e360.
2. Songyang Z. Introduction to Telomeres and Telomerase. Vol 1587. New York, NY: Humana Press; 2017.
Telomere Length and Vascular Phenotypes

16. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Masi S, D’Aiuto F, Martin-Ruiz C, Kahn T, Wong A, Ghosh AK, Whincup P, Kuh D, Hughes A, Zglinicki TC, Hardy R, Deanfield JE. Rate of telomere shortening and cardiovascular damage: a longitudinal study in the 1946 British Birth Cohort. Eur Heart J. 2011;32:148–149.

17. Fitzpatrick AL, Kronal RA, Gardner JP. Leukocyte telomere length and cardiovascular disease in the Cardiovascular Health Study. Am J Epidemiol. 2007;165:14–21.

18. Masi S, D’Auito F, Martin-Ruiz C, Kahn T, Wong A, Ghosh AK, Whincup P, Kuh D, Hughes A, Zglinicki TC, Hardy R, Deanfield JE. Rate of telomere shortening and cardiovascular damage: a longitudinal study in the 1946 British Birth Cohort. Eur Heart J. 2011;32:148–149.

19. De Meyer T, Rietzschel ER, De Buyzere ML, Langlois MR, De Bacquer D, Segers P, Van Damme P, De Backer GG, Van Oostveldt P, Van Credik W, Gillebert TC, Bekaert S, Asklepios Study Investigators. Systemic telomere length and preclinical atherosclerosis: the Asklepios study. Eur Heart J. 2009;30:3074–3081.

20. Fernández-Alvira JM, Fuster V, Dorado B, Soberón N, Flores I, Gallardo M, Pocock S, Blasco MA, Andrés V. Short telomere load, telomere length, and subclinical atherosclerosis: the PESA study. J Am Coll Cardiol. 2016;67:2467–2476.

21. Wang YY, Chen AF, Wang HZ, Xie LY, Sui KK, Zhang QY. Association of shorter mean telomere length with large artery stiffness in patients with coronary heart disease. Aging Male. 2011;14:27–32.

22. McDonnell BJ, Yasmin Butler J, Cockcroft JR, Wilkinson IB, Erusalimsky JD, McEvoy CM. The age-dependent association between aortic pulse wave velocity and telomere length. J Physiol. 2017;595:1627–1635.

23. Thijssen DH, Carter SE, Green DJ. Arterial structure and function in vascular ageing: are you as old as your arteries? J Physiol. 2016;594:2275–2284.

24. Wake M, Clifford S, York E, Davies S; Child Health Checkpoint Team. Introducing growing up in Australia’s Child Health Checkpoint. Fam Matters. 2014;94:15–23.

25. Clifford S, Davies S, Wake M. Child Health Checkpoint: cohort summary and methodology of a physical health and biospecimen module for the Longitudinal Study of Australian Children. BMJ Open. 2019;0:e020261.

26. Edwards B. Growing up in Australia: the Longitudinal Study of Australian Children entering adolescence and becoming a young adult. Fam Matters. 2014;95:5–14.

27. Sanson A, Johnstone R; The LSAC Research Consortium & Facs LSAC Project Team. Growing up in Australia takes its first steps. Fam Matters. 2004;67:46–53.

28. Kies E, Cseprekai O, Kerti A, Salvi P, Benetos A, Tisler A, Szabo A, Tulasay A, Reusz GS. Measurement of pulse wave velocity in children and young adults: a comparative study using three different devices. Hypertens Res. 2011;34:1197–1202.

29. Selamet Tierney ES, Gauvreau K, Jaff MR, Gal D, Nourse SE, Trevey S, O’Neill S, Baker A, Newburger JW, Colan SD. Carotid artery intima-media thickness measurements in the youth: reproducibility and technical considerations. J Am Soc Echocardiogr. 2015;28:301–316.

30. Papaioannou TG, Karatzis EN, Karatzi KN, Galafos EJ, Pr catalyst on AD, Stamatakis ES, Papamichalis CM, Lekakis JP, Stefanidis CI. Hour-to-hour and week-to-week variability and reproducibility of wave reflection indices derived by aortic pulse wave analysis: implications for studies with repeated measurements. J Hypertens. 2007;25:1678–1686.

31. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30:447.

32. Growing Up in Australia’s Child Health Checkpoint: Standard Operating Procedure: Telomere Length Quantification. Melbourne, Australia: Murdoch Children’s Research Institute; 2018.

33. Nguyen MT, Lycett K, Vryer R, Burgner D, Ranganathan S, Grobler A, Wake M, Saffery R. Telomere length: population epidemiology and concordance in 11–12 year old Australians and their parents. BMJ Open. 2019;0:e020263.

34. Liu RS, Dunn S, Grobler A, Lange K, Becker D, Goldsmith G, Carlin J, Juonala M, Wake M, Burgner DP. Carotid artery intima-media thickness, distensibility, and elasticity: population epidemiology and concordance in Australian 11–12 year old Australians and their parents. BMJ Open. 2019;0:e020264.

35. Kahn F, Wake M, Lycett K, Clifford S, Burgner D, Goldsmith G, Grobler A, Lange K, Cheung M. Vascular function and stiffness: population epidemiology and concordance in Australian 11–12 year-olds and their parents. BMJ. 2019;0:mm2089.

36. Janner JH, Godtfredsen NS, Ladelsund S, Vestbo J, Prescott E. Aortic atherogenesis index: reference values in a large unselected population by means of the SphygmoCor device. Am J Hypertens. 2010;23:180–185.

37. Strazhiskos I, Tkacheva O, Boyskov S, Akasheva D, Dudinskaya E, Vygodin V, Skvortsov D, Nilsson P. Association of insulin resistance, arterial stiffness and telomere length in adults free of cardiovascular diseases. PLoS One. 2015;10: e0136676.

38. Butlin M, Qasem A, Battista F, Bozec E, McEniery CM, Millet-Amaury E, Pucci G, Wilkinson IB, Schillaci G, Bouteury P, Avolio AP. Cardiot-femoral pulse wave velocity assessment using novel cuff-based techniques: comparison with tonometric measurement. J Hypertens. 2013;31:2237–2243; discussion 2243.

39. Wang MH, Yoo JK, Kim HK, Wang CL, Mackay K, Hemstreet O, Nichols WW, Christou D. Validity and reliability of aortic pulse wave velocity and atherogenesis index determined by the new cuff-based SphygmoCor Xcel. J Hum Hypertens. 2014;28:475–481.

40. Wilkinson IB, McEvoy CM, Schillaci G, Bouteury P, Segers P, Donald A, Chomczynsky PJ. ARTERY Society guidelines for validation of non-invasive haemodynamic measurement devices: part 1, arterial pulse wave velocity. Artery Res. 2010;3:34–40.

41. Butlin M, Qasem A. Large artery stiffness assessment using SphygmoCor technology. Pulse (Basel). 2017;4:180–192.

42. Mundstock E, Sarría EE, Zatti H, Louzada F, Grun L, Jones M, Guma F, Memori M, Epifanio M, Stein RT, Barbe-Tuana FM, Mattioli R. Effect of obesity on telomere length: systematic review and meta-analysis. Obesity. 2015;23:2165–2174.

43. Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J, Garfinkel I, Nettleson D. Social disadvantage, genetic sensitivity, and children’s telomere length. Prog Natl Acad Sci USA. 2014;111:5944–5949.
44. Astuti Y, Wardhana A, Watkins J, Wulaningsih W, Network PR. Cigarette smoking and telomere length: a systematic review of 84 studies and meta-analysis. Environ Res. 2017;158:480–489.

45. Kuczynska RJ, Ogden CL, Grummer-Strawn LM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson C. CDC growth charts: United States. Adv Data. 2000;314:1–28.

46. Ellul E, Wake M, Clifford S, Lange K, Wurz P, Juonala M, Dwyer T, Carlin J, Burgner D, Saffery R. Metabonomics: population epidemiology and concordance in 11-12-year-old Australians and their parents. BMJ Open. 2019;9:e020900.

47. Kalisch DW. National Health Survey: First Results. Canberra: Australian Bureau of Statistics; 2015.

48. Okuda K, Khan MY, Skurnick J, Kimura M, Aviv A. Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. Atherosclerosis. 2000;152:391–398.

49. Wilson WR, Herbert KE, Mistry Y, Stevens SE, Patel HR, Hastings RA, Thompson MM, Williams B. Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. Eur Heart J. 2008;29:2689–2694.

50. O’Donnell CJ, Demissie S, Kimura M, Levy D, Gardner JP, White C, D’Agostino RB, Wolf PA, Polak J, Cupples LA, Aviv A. Leukocyte telomere length and carotid artery intimal medial thickness: the Framingham Heart Study. Arterioscler Thromb Vasc Biol. 2008;28:1165–1171.

51. Willeit P, Willeit J, Brandstätter A. Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. Arterioscler Thromb Vasc Biol. 2010;30:1649–1656.

52. Zhang W, Zhu S, Zhao D, Jiang S, Li J, Li Z, Fu B, Zhang M, Li D, Bai X, Cai G, Sun X, Mei Z, Curtin LR, Roche AF, Johnson C. Telomere length predicts advanced atherosclerosis and cardiovascular disease risk. Arterioscler Thromb Vasc Biol. 2014;34:843–850.

53. Regin G, Panatta E, Candi E, Melino G, Amelio I, Balistreri CR, Annicchiarico-Petruszelli M, Di Daniele N, Ruvolo G. Vascular ageing and endothelial cell senescence: molecular mechanisms of physiology and diseases. Mech Ageing Dev. 2016;159:14–21.

54. Dudinskaya EN, Tkacheva ON, Shestakova MV, Brailova NV, Strazhsko ID, Akasheva DJ, Isaykina OH, Sharashkina NV, Kashkanova DA, Boytsov SA. Short telomere length is associated with arterial aging in patients with type 2 diabetes mellitus. Endocr Connect. 2015;4:136–143.

55. Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A. Telomere length as an indicator of biological aging. Hypertension. 2001;37:381–385.

56. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Rulope LM, Schmieder RE, Simes PA, Sleight P, Voruganti M, Waeber B, Zannad F, Redon J, Dominczak A, Narkiewicz K, Nilsson PM, Burnier M, Viigimaa M, Ambrosoni E, Caufield M, Coca A, Olsen MH, Schmieder RE, Tsoufis C, van de Borne P, Zamorano JL, Aschenbach B, Baumgartner B, Bysshe J, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knudt J, Kolh P, Lancellotti P, Linhart A, Nikoyannopulos P, Piypoli BF, Sirnes L, Tamargo JL, Tendera M, Torblicki A, Wijns W, Wissekerber C, Clement DL, Coca A, Gillebert TC, Tendera M, Roei EA, Ambrosoni E, Anker SD, Bauersachs J, Hatt J, Caufield M, De Buyzere M, Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germao G, Giezen S, Haller H, Hoes AW, Jordan J, Kahan T, Komajda M, Duvic M, Mahrholdt H, Olsen MH, Osterger J, Parati G, Perk J, Polonia J, Popses VA, Reiner Z, Ryden L, Sirekno Y, Stnton A, Struijker-Boudier H, Tsoufis C, van de Borne P, Vladopulos C, Volpe M, Wood DA. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34:2159–2219.

57. Dalla Pozza R, Ehring-Schelitska D, Fritsch P, Jokinen E, Petrokassas A, Oberhoffer R; Association for European Paediatric Cardiology Working Group Cardiovascular Prevention. Intima media thickness measurement in children: a statement from the Association for European Paediatric Cardiology (AEPc) Working Group on Cardiovascular Prevention endorsed by the Association for European Paediatric Cardiology. Atherosclerosis. 2015;238:380–387.
Supplemental Methods

Blood collection and genomic DNA isolation

Whole venous blood was collected into vacutainer tubes containing ethylenediaminetetraacetic acid (known as EDTA) and immediately transported to an on-site laboratory. The blood sample was processed into aliquots within 2 hours into 1.0 mL FluidX tubes (FluidX, Cheshire, UK) and frozen in a -80 °C ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, USA). Samples were transported to the Murdoch Children’s Research Institute (MCRI) biobank. Genomic DNA was isolated from available blood (e.g. whole blood or blood clot) using the Qiaamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Samples were randomised with child and parent dyads on the same plate to minimise batch effects using a random number generator (Stata 14.2, StataCorp LLC, USA). The sample retrieval, protocol optimisation, consumable acquisition, and isolation of genomic DNA spanned April 2016 to January 2017. Purity and integrity of genomic DNA was confirmed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Middleton, USA), Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, USA) and gel electrophoresis, prior to storage in a -80 °C ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA was also isolated from 3 sets of control samples: (1) the K562 leukemic cell line, (2) newborn cord blood and (3) human placental tissue. These control samples have previously been described as having ‘shorter’, ‘average’ and ‘longer’ telomeres relative to peripheral blood samples.1-4 Genomic DNA from each of these control samples was used on all telomere assays to assess day-to-day and plate effects.

Telomere length measurement

Each sample was measured in quadruplicates comprising 4 μl of diluted genomic DNA at 5 ng/μl, 5 μl of SensiFAST SYBR No-ROX Kit master mix (Bioline, Sydney, Australia) and 0.5 μl of each forward and reverse primer at 2 μM. The primer sequences were tel1 (5’-CGG TTT GGT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT), tel2 (5’-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT), bg1 (5’-GCA GGA GCC AGG GCT GGG CAT AAA AGT CA) and bg2 (5’-GGG CCT CAC CAC CAA CTT CAT CCA CGT TC). All ‘T’ and ‘S’ reactions were performed in 384-well plates on a Lightcycler 480 Instrument II (Roche, Melbourne, Australia). The cycling condition began with incubation at 95°C for 10 minutes, followed by 35 cycles of (i) 95°C for 15 seconds and (ii) 62°C for 60 seconds. The final 384-well layout included participant genomic DNA, three sets of control genomic DNA and a no-template control containing RNase-free water instead of a genomic DNA template, each present in quadruplicates. Further details are described in the standard operating procedure on the Growing Up in Australia’s CheckPoint website.5

Calculation of telomere length

A ratio, known as the T/S ratio, is calculated by comparing the relative amount of ‘T’ and ‘S’ for each of these samples to a reference genomic DNA sample (in this case the average T/S ratio of all control genomic DNA). The final relative telomere length from each sample, based on the T/S ratio, was calculated as the change in cycle threshold (Ct) of the test sample (Table S1), normalised to the average T/S ratio of the control genomic DNA on the corresponding plate (Table S2). Hence, the final equation is shown in Table S3. If less than two successful replicates out of the quadruplicates for either ‘T’ and ‘S’ were measured then the sample data was omitted (n=16), otherwise a median was calculated, resulting in a median ‘T’ and a median ‘S’ for each sample.
A Ct replicate from 5 to 28 was considered successful as a Ct outside of this range was uncertain.

**Telomere length replicate variability**

To assess the replicate reliability, the degree of variation between replicates in the assay plate, an intra-assay coefficient of variation was calculated. The intra-assay coefficient of variation was calculated as the ratio of the pooled Ct's standard deviation (SD) from all samples (each was analysed in quadruplicate) and the overall Ct mean, and then multiplied by 100. To assess the degree of assay-to-assay and day-to-day consistency an inter-assay coefficient of variation was calculated using the pooled Ct's SD divided by the overall Ct mean of all duplicated samples, and then multiplied by 100. The mean intra-assay variability between ‘T’ and ‘S’ quadruplicates used in the calculation of the T/S ratio was 1.7% (SD 0.3; range 0.9-2.6). The inter-assay variability between plates was 1.7% (SD 1.4; range 0.3-6.2).

**Reliability of carotid intima-media thickness (IMT) readings**

Six trained raters analysed all cine-loops. Training consisted of thirty example cine-loops that were subsequently assessed for consistency by an expert rater. Inter- and intra-observer variability was assessed by reanalysing a subset of 105 randomly-selected child images four times at the end of the scoring process. Images were reassessed twice each by two raters in a balanced incomplete block design as not all raters assessed the complete subset. This allowed estimation of the repeatability of measurements made by the same rater and the reproducibility of measurements made by different raters. Image acquisition was only performed once.

In our reliability analysis, we used the modelling of repeated measurements on carotid IMT films with rater and participant as random effects to estimate between-participant variance, between-rater variance, and residual error variance. These were used to calculate intra- and inter-observer variability (the degree of measurement error proportional to the mean.) For maximum carotid IMT value, the intra-observer variability was 4.9% (95% CI 4.6-5.2) and the inter-observer variability was 6.2% (95% CI 5.2-7.2).

**Reliability of carotid elasticity**

Carotid elasticity measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation. Carotid elasticity was calculated from carotid artery images and expressed as a percentage change in intima-intima lumen diameter per change in pulse pressure (Table I), according to previously published work from the Cardiovascular Risk in Young Finns Study and other related studies. Intima-intima lumen diameter measurement was automated using Carotid Analyser (Medical Imaging Applications, Coralville, IA, USA), rater-independent, and was calculated by measuring the average intima-intima distance (subtracting near and far wall IMT measurements) on each of the three to five still frames used to calculate maximum carotid IMT. The final calculation is shown in Table S1 (IV).

After algorithmic detection of the intima-media interface over the entire cine-loop by Carotid Analyser (Medical Imaging Applications, Coralville, IA, USA), frames were manually adjusted as needed or rejected if the intima-media interface was unclear or blurred. Intra- and inter-observer variability for maximum lumen diameter was 1.3% (95% CI 1.2-1.4) and 1.6% (95% CI 1.4-1.9). Intra- and inter-observer variability for minimum lumen diameter was 1.2% (95% CI 1.1-1.3) and 1.5% (95% CI 1.2-1.7).

**Reliability of arterial pulse waveforms**

Twenty children and twenty parents were randomly sampled from the SphygmoCor database and sampling was stratified by two analysts. Hence, twenty subjects were chosen from each analyst. The pulse wave analysis (PWA) waveforms had already undergone
quality checks by one of these two trained data analysts. Each child provided three individually recorded PWA and whilst temporally close they were not necessarily exchangeable and were treated as linked for rating purposes. Waves were presented to the analyst without evidence of previous quality assessment. Three waves were present for 32 children and two waves for 8 children, resulting in each analyst making 112 assessments. Waves from the same subject were identifiable as they were presented sequentially for assessment. To assess the quality of the PWA waveforms we compared the quality ratings (1 good, 2 adequate and 3 poor) assigned by each analyst.

We compared quality ratings of the PWA waveforms by calculating the proportion of positive and negative agreement between analysts. No waves were classified as being of poor quality. The positive agreement between analysts was high (0.99). There were discrepancies in quality ratings for only two waveforms and therefore the negative agreement (0.5) was likely a poor estimate. Overall both analysts agreed that all scans were of acceptable quality or above.
Table S1. Equations for the calculation of (I, II and III) telomere length and (IV) carotid elasticity.

| Equation | Description |
|----------|-------------|
| (I) Change in cycle threshold for test sample | \( \Delta C_t^{\text{test}} = C_t^{(\text{test, telomere})} - C_t^{(\text{test, beta-globin})} \) |
| (II) Change in cycle threshold for reference sample | \( \Delta C_t^{\text{ref}} = C_t^{(\text{ref, telomere})} - C_t^{(\text{ref, beta-globin})} \) |
| (III) Relative telomere length (T/S ratio) | \( \frac{2^{\Delta C_t}}{2^{\Delta C_t^{\text{test}}} - 2^{\Delta C_t^{\text{ref}}}} \) |
| (IV) Carotid elasticity (%/mmHg) | \( \left( \frac{L_D^{\text{max}} - L_D^{\text{min}}}{L_D^{\text{min}}} \right) \left( \frac{\Delta P}{\Delta P} \right) \times 100\% \) |
## Table S2. Summary of adults’ vascular outcomes for diabetics, current smokers and hypertension medications users.

|                        | Carotid IMT (µm) | Carotid-femoral PWV (m/s) | Carotid elasticity (% per 10 mmHg) |
|------------------------|------------------|---------------------------|------------------------------------|
|                        | N    | Mean (SD) | p-value* | N    | Mean (SD) | p-value* | N    | Mean (SD) | p-value* |
| **Diabetes**           |      |           |          |      |           |          |      |           |          |
| Yes                    | 30   | 758 (170) | -        | 24   | 7.81 (1.39)| -        | 22   | 2.05 (0.63)| -        |
| No                     | 1286 | 661 (93)  | 0.004    | 1176 | 6.94 (1.11)| 0.006    | 1105 | 2.44 (0.60)| 0.008    |
| **Current smoker**     |      |           |          |      |           |          |      |           |          |
| Yes                    | 110  | 658 (91)  | -        | 94   | 6.78 (1.08)| -        | 94   | 2.43 (0.62)| -        |
| No                     | 1206 | 664 (97)  | 0.51     | 1106 | 6.97 (1.12)| 0.11     | 1033 | 2.44 (0.60)| 0.95     |
| **Hypertension medication** |    |           |          |      |           |          |      |           |          |
| Yes                    | 66   | 720 (124) | -        | 55   | 8.00 (1.40)| -        | 52   | 2.13 (0.50)| -        |
| No                     | 1250 | 660 (94)  | 0.0002   | 1145 | 6.91 (1.08)| <0.0001  | 1075 | 2.45 (0.60)| <0.0001  |

SD: standard deviation.
* Student’s t-test.

Downloaded from http://ahajournals.org by on June 11, 2019.
Table S3. Summary of adult T/S ratios for diabetics, hypertension current smokers and medication users.

|                          | Telomere length (T/S ratio) |   |   |
|--------------------------|-----------------------------|---|---|
|                          | N  | Mean (SD) | p-value* |
| Diabetes                 |   |   |   |
| Yes                      | 32 | 0.76 (0.30) | - |
| No                       | 1312 | 0.81 (0.38) | 0.40 |
| Current smoker           |   |   |   |
| Yes                      | 110 | 0.77 (0.34) | - |
| No                       | 1234 | 0.81 (0.38) | 0.20 |
| Hypertension medication  |   |   |   |
| Yes                      | 68 | 0.78 (0.36) | - |
| No                       | 1276 | 0.81 (0.38) | 0.48 |

SD: standard deviation.

* Student's t-test.
Table S4. Association of brachial systolic blood pressure (exposure) with telomere length (separately, outcome), in children and adults per 10 mmHg

| Outcome  | Children | Adults |
|----------|----------|--------|
|          | N     | Coefficient (95% CI) | p-value | N     | Coefficient (95% CI) | p-value |
| T/S ratio | 1140  | -0.02 (-0.06 to 0.02) | 0.25    | 1141  | -0.02 (-0.04 to -0.002) | 0.03    |
| T/S ratio | 1139  | -0.002 (-0.04 to 0.04) | 0.92    | 1136  | -0.02 (-0.04 to -0.002) | 0.03    |

* Model 1 adjusted for sex, age and sample type.
† Model 2 additionally adjusted for body mass index, Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage score, plus smoking status for adults.
Figure S1. Children's (green, solid) and adults' (blue, dash) distribution of (I) carotid intima-media thickness, (II) carotid-femoral pulse wave velocity, (III) carotid elasticity and (IV) telomere length.
Supplemental References:

1. Akiyama M, Yamada O, Kanda N, Akita S, Kawano T. Telomerase overexpression in K562 leukemia cells protects against apoptosis by serum deprivation and double-stranded DNA break inducing agents, but not against DNA synthesis inhibitors. *Cancer Lett.* 2002;178:187-197.

2. Allsopp R, Shimoda J, Easa D, Ward K. Long telomeres in the mature human placenta. *Placenta.* 2007;28:324-327.

3. Martens DS, Plusquin M, Gyselaers W, Vivo I, Nawrot TS. Maternal pre-pregnancy body mass index and newborn telomere length. *BMC Med.* 2016;14:1-10.

4. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. *Pediatr Res.* 2002;52:377-381.

5. Growing Up in Australia's Child Health CheckPoint. Standard Operating Procedure: Telomere Length Quantification. *Melbourne: Murdoch Children's Research Institute.* 2018.

6. Juonala M, Jarvisalo MJ, Maki-Torkko N, Kahonen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation.* 2005;112:1486-1493.

7. Marlatt KL, Kelly AS, Steinberger J, Dengel DR. The influence of gender on carotid artery compliance and distensibility in children and adults. *J Clin Ultrasound.* 2013;41:340-346.