Published in final edited form as:

Atherosclerosis. 2017 November ; 266: 41–47. doi:10.1016/j.atherosclerosis.2017.09.016.

Leukocyte telomere length and cardiovascular disease in African Americans: The Jackson Heart Study

Stanford Mwasongwe^{a,*}, Yan Gao^{b,*}, Michael Griswold^c, James G. Wilson^b, Abraham Aviv^d, Alexander P. Reiner^e, and Laura M. Raffield^f

^aJackson Heart Study, School of Public Health, Jackson State University, Jackson, MS, USA

^bDepartment of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

^cDepartment of Data Science, John D Bower School of Population Health, University of Mississippi Medical Center, Jackson, MS, USA

^dCenter of Human Development and Aging, New Jersey Medical School, Rutgers, Newark, NJ, USA

^eDepartment of Epidemiology, University of Washington, Seattle, WA, USA

^fDepartment of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Background and aims—In European descent populations, shorter leukocyte telomere length (LTL) has been associated with subclinical atherosclerosis, cardiovascular disease (CVD), and mortality, while longer LTL has been associated with greater left ventricular hypertrophy. We evaluated the relationship of LTL with subclinical cardiovascular disease indices and incident clinical events and mortality in African Americans (AAs).

Methods—Analyses were restricted to 2,518 participants of the Jackson Heart Study (JHS) with LTL measured by Southern blot in baseline blood samples.

Results—Adjusting for established CVD risk factors, longer LTL was significantly associated with lower prevalence of coronary artery calcification (CAC) (odds ratio (OR) =0.810 per 1 kb increase in LTL; (95% confidence interval [CI] 0.656, 0.9998), $p=0.0498$). Longer LTL was also associated with higher ankle brachial index (ABI) ($\beta=0.023$; (95% CI 0.004, 0.042), $p=0.017$).

ADDRESS FOR CORRESPONDENCE: Department of Genetics, University of North Carolina, 5100 Genetic Medicine Building, 120 Mason Farm Road, Chapel Hill, NC, 27599, United States. laura_raffield@unc.edu (L. M. Raffield).

*These authors contributed equally to this work as first authors

CONFLICT OF INTEREST

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

AUTHOR CONTRIBUTIONS

SM and LMR interpreted the data analysis and wrote the manuscript. YG analyzed and interpreted the data and critically reviewed the manuscript. MG helped design the data analysis and critically reviewed the manuscript. AA, JGW, and APR obtained funding, designed the study, and critically reviewed the data analysis and manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

when comparing the highest to the lowest LTL quartile. There were no significant associations between LTL and abdominal aortic calcification, carotid intima-media thickness, or left ventricular mass. After a median follow-up of 9 years, longer LTL was associated with lower risk of incident ischemic stroke (hazard ratio (HR) 0.69 (95% CI 0.48, 0.99), $p=0.044$) and total mortality (HR 0.81 (95% CI 0.67, 0.97), $p=0.026$) in age and sex adjusted models, but these associations were no longer significant in fully adjusted models.

Conclusions—Among a community-based cohort of AAs, longer LTL was nominally associated with lower odds of CAC and increased ABI, indicative of decreased prevalence of subclinical atherosclerosis and peripheral arterial disease. These findings do not offer strong support for LTL as an independent biomarker of CVD risk in AAs

Keywords

coronary artery calcification; telomeres; coronary heart disease; stroke; mortality; ankle-brachial index

INTRODUCTION

Telomeres, the DNA-protein structures located at the end of chromosomes, progressively shorten with successive cell divisions. Telomere shortening eventually triggers replicative senescence in cultured somatic cells, and possibly tissue and organismal aging *in vivo*. Many studies have examined telomere length dynamics (telomere length and/or attrition) as a potential biomarker of biological aging and predictor of aging-related diseases [1]. Shorter leukocyte telomere length (LTL) has been associated with higher risk of mortality [2, 3] and cardiovascular disease (CVD) events [3–5]. LTL is relatively stable during adulthood, suggesting that the links between LTL and health status may be established early in life [6]. Genetic risk scores of variants associated with shorter telomere length have been associated with increased risk of coronary artery disease events [7, 8], suggesting a potential causative role for telomere length, as expressed in LTL, in CVD and other age-related diseases. Besides clinical CVD, associations have also been observed between shorter LTL and various subclinical indices of atherosclerosis burden, such as coronary artery calcification (CAC) [9, 10] and carotid intima-media thickness (cIMT) [11]. By contrast, longer LTL has been associated with greater left ventricular hypertrophy [12, 13].

Along with chronologic age, various demographic and environmental factors such as smoking [3, 14, 15], male sex [3], younger paternal age at conception [16], lower socioeconomic status [17], sedentary lifestyle and higher body mass [14, 15, 18], and genetic factors [7, 19, 20] can contribute to variation in telomere length. Despite the higher rates of CVD and CVD mortality among African American (AA) compared to European American (EA) populations [21], AAs have been shown to have longer LTL compared to EAs [2, 10, 22]. AAs also display different patterns of subclinical vascular disease, including less atherosclerosis (as measured by coronary artery calcium) [23] but more LVH than EAs [24]. Despite these potentially important inter-ethnic differences in CVD risk and subclinical disease, data are limited on the connection between LTL and CVD in AAs; to our knowledge, no study has assessed associations of LTL with both multiple subclinical CVD measures and CVD events in this population. In two studies the associations of shorter LTL

with subclinical atherosclerosis [10] or incident CVD and mortality risk [25] observed in EAs were not present in AAs.

To further address these questions, we examined the relationship of LTL with risk of CVD, assessed using subclinical indices (CAC, abdominal aortic calcification [AAC], cIMT, ankle-brachial index [ABI], left ventricular [LV] mass and LV mass index [LVMI]) and incident clinical events (coronary heart disease [CHD], stroke, and heart failure hospitalizations) and mortality in AAs from the Jackson Heart Study (JHS).

MATERIALS AND METHODS

Sample population and LTL measurements

The JHS is a prospective, community-based cohort designed to investigate risk factors for cardiovascular disease among AAs of the Jackson, Mississippi, metropolitan tri-county area (Hinds, Madison, and Rankin). The design and methods of this study have been previously described [26]. At baseline (September 2000 – March 2004), a total of 5,306 AAs 21 years of age were recruited to participate in the study from four pools: random sampling (17% of participants), volunteers (30%), participants in the Atherosclerosis Risk in Communities (ARIC) study (31%), and secondary family members (22%). After completing a baseline clinic visit (Exam 1), participants returned for two additional clinic visits, Exam 2 (October 2005 – December 2008) and Exam 3 (February 2009 – January 2013). All participants included in this analysis provided written, informed consent for use of genetic data, and all study protocols conform to the 1975 Declaration of Helsinki guidelines. The study was approved by the Institutional Review Boards of the participating institutions (University of Mississippi Medical Center, Jackson State University and Tougaloo College).

All participants were asked to provide blood samples at the baseline clinic visit. DNA was extracted from whole blood using Puregene reagents (Gentra System, Minneapolis, MN) [27]. LTL (in kilobases) was measured at the Center of Human Development and Aging at the New Jersey Medical School, Rutgers, using the Southern blot analysis method [28]. Quality control consisted of assessment of DNA integrity prior to LTL measurement. The average inter-assay coefficient of variation was 2.0%; for individual measures of LTL, the interclass correlation coefficient was 0.95. Of 2,573 participants with genomic DNA, 2,518 (61.0% female) had non-missing LTL values with adequate DNA content and were eligible for analysis. Participants with non-missing LTL values were slightly less likely to be female than the cohort as a whole (63.5% female, $p=0.001$), but did not differ significantly in age ($p=0.63$).

Covariates

Trained interviewers administered standard questionnaires to assess demographic characteristics (age, sex, and education), medical history, medication use, and lifestyle and health behavior (alcohol consumption, current smoking, and physical activity). Anthropometric measures and fasting blood and urine samples were collected following standardized procedures. Lipid profiles (total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL]), and hemoglobin A1c (HbA1c) were assessed at the

University of Minnesota, while blood cell traits (white blood cell [WBC], red blood cell [RBC] and platelet count), plasma glucose, and serum creatinine were measured at the University of MS Medical Center.[27] Prevalent diabetes was defined according to the American Diabetes Association (ADA) criteria as fasting glucose ≥ 126 mg/dL, HbA1c $\geq 6.5\%$, or self-reported use of a diabetes medication within 2 weeks prior to the clinic visit. Hypertension was defined as systolic blood pressure above 140 mmHg, diastolic blood pressure over 90 mmHg, or use of antihypertensive medications. Glomerular filtration rate (eGFR) was estimated from serum creatinine using the CKD-EPI equation [29]. Self-reported physical activity was assessed as a dichotomous measure using American Heart Association (AHA) criteria for ideal health; participants were grouped as having either poor health (no activity) or intermediate/ideal health (some reported physical activity) [30].

Subclinical cardiovascular disease

Computed tomography (CT), ultrasound, and echocardiographic imaging data collection, reading, and quality control in JHS have been previously described [26, 27, 31]. Using B-mode ultrasonography, cIMT was quantified as the maximum likelihood estimate of average right and left common carotid far intima-media thickness [32]. For the present study, LVM was calculated using the American Society of Echocardiography corrected formula by Devereux et al [33]. LVM was divided by height in meters to the exponential of 2.7 to normalize heart to body size while LVMI was calculated as LVM/body surface area. Using Doppler ultrasound, JHS staff measured systolic blood pressure (mmHg) at the posterior tibial artery in the leg and the brachial artery in the arm. Ankle brachial index (ABI) was defined as the ratio of systolic blood pressure (SBP) of the posterior tibial artery to that of the brachial artery [34]. Individuals with an ABI > 1.4 ($n=84$) were excluded.

Cardiovascular imaging for coronary and aortic calcification was conducted at Exam 2 (median interval between Exam 1 and 2: 4.5 years; interquartile range 4.3–5.0 years). CT of the heart and lower abdomen was performed using a Lightspeed 16 Pro 16-channel multidetector system equipped with cardiac gating (GE Healthcare, Milwaukee, WI). Quality control and image analysis were performed at the JHS core reading center at Wake Forest University School of Medicine. CT scans of the coronary arteries were based on standard protocols developed for the MESA and CARDIA studies [35]. CAC and AAC were measured in CT images by trained and experienced technologists. The Agatston score [36], modified to account for slice thickness, was used to quantify calcified artery plaque, computed by multiplying the area of each lesion by a weighted attenuation score (in Hounsfield units) on a TeraRecon Aquarius Workstation. Reproducibility of CAC and AAC was 0.99. Presence of CAC or AAC was defined as Agatston score > 0 .

Incident cardiovascular disease and mortality

Evaluation of adjudicated incident CHD, stroke, and HF events was performed through December 31, 2012, whereas all-cause mortality includes all deaths through December 31, 2010. Adjudication procedures for CHD, HF, and stroke have been described previously [37]. Briefly, hospital discharge lists with relevant International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) codes were obtained from the Jackson, Mississippi tri-county area hospitals and out of catchment area hospitals as necessary.

Potential CVD and vital status were also assessed by contacting living participants or their proxies annually via telephone. Mortality was assessed through death certificates requested from the Mississippi State Department of Health and National Death Index searches. When CVD-related hospitalizations were identified, medical records were retrieved and abstracted. Trained clinicians adjudicated events as described previously [37]. For the current analysis, a CHD event was defined as fatal or nonfatal MI or coronary revascularization; hard CHD excluded coronary revascularization, including only MI and fatal CHD. Heart failure (HF) was not assessed at baseline; incident HF included probable or definite HF hospitalization or death from 2005–2012. Stroke was defined as embolic or thrombotic brain infarction, brain hemorrhage or subarachnoid hemorrhage. Participants were followed up for a median of 8 years for HF, 10 years for CHD and stroke, and 9 years for all-cause mortality. All participants with a history of CHD or stroke were excluded from those incident event analyses, with participants with a HF hospitalization between Exam 1 and the start of 2005 or a self-reported history of heart failure also excluded from incident HF hospitalization analyses.

Statistical analysis

Quartiles of LTL were derived (quartile (Q) 1, mean 6.33 kb, Q2, mean 6.93 kb, Q3, mean 7.38 kb, and Q4, mean 8.08 kb). Trends across quartiles were assessed by including quartiles of LTL as an ordinal variable in the generalized linear models setting proper link functions for all demographic factors and subclinical CVD measures, unadjusted and adjusted for age and sex. LVM, LVMI, and cIMT, along with hsCRP and triglycerides, were natural log-transformed to obtain normal distributions. Next, associations of LTL as a continuous measure with subclinical CVD measures were assessed. Linear regression models were used for the continuous normal-distributed outcomes, and logistic regression models were used for the dichotomous measures (presence or absence of CAC and AAC). Tobit regression models were used to assess the associations between LTL and log transformed CAC and AAC due to left censoring. As a supplemental analysis, associations of each LTL quartile in reference to the lowest quartile were assessed for subclinical CVD measures. Cox proportional hazards regression was used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) for incident CVD events (CHD, HF, stroke) or all-cause mortality. The following sequential models were estimated for subclinical and incident CVD measures: Model 1, adjusted for age and sex; Model 2, adjusted for BMI and current smoking status; and Model 3, with additional adjustments for commonly assessed CVD risk factors including SBP, diabetes and LDL. *P*-values less than 0.05 were considered statistically significant. Confidence intervals (CIs) are expressed as 95% CIs. All analyses were performed in STATA version 14 (StataCorp, College Station, Texas).

RESULTS

Demographic and clinical characteristics of JHS participants, as well as summary statistics for subclinical CVD measures, are presented in Table 1, both for the whole sample and stratified by LTL quartile. All measures were assessed at Exam 1 (2000–2004), with the exception of CAC and AAC measures, which were assessed at Exam 2 (2005–2008). Table 1 also displays the *p*-values for age- and sex- adjusted models assessing trend across LTL

quartiles for demographic and clinical characteristics and subclinical CVD measures. As anticipated, female sex and younger age were each strongly associated with longer LTL ($p<0.0001$). Associations with additional demographic and clinical characteristics and unadjusted models are presented in Supplemental Table 1. In age- and sex-adjusted models, current smoking was the only other characteristic significantly associated with shorter LTL ($p=0.014$).

Associations of LTL with subclinical CVD are further explored in Table 2, with models assessing LTL as a continuous predictor variable for outcomes of subclinical CVD measures. Adjusting for age and sex (Model 1), longer LTL was significantly associated with lower CAC score and lower odds of presence of CAC. In models additionally adjusting for well-established CVD risk factors (Models 2 and 3), the associations between LTL and CAC were attenuated. In the most fully adjusted model (Model 3, adjusted age, sex, BMI, current smoking, SBP, diabetes, and LDL), the odds ratio (OR) for presence of CAC was 0.810 per 1 kb increase in LTL; 95% confidence interval [CI], 0.656, 0.9998; $p=0.0498$. There were no other significant associations with subclinical CVD measures in fully adjusted models.

As a supplemental analysis for subclinical CVD measures, we also modeled the LTL predictor variable categorically, comparing the top (mean 8.08 kb, SD 0.36) and bottom quartiles (mean 6.33 kb, SD 0.30) of LTL. (Supplemental Table 2). The association results for CAC in Models 1, 2, and 3 were qualitatively similar to those obtained above for LTL as a continuous predictor variable. In addition, the top quartile of LTL compared to the bottom was associated with higher ABI (Model 3, $\beta=0.023$, 95% CI 0.004, 0.042, $p=0.017$) when fully adjusted for CVD risk factors. However, none of these associations with subclinical CVD traits meet a Bonferroni corrected p -value threshold for six tested traits ($0.05/6=p<0.008$).

We next assessed association of LTL with mortality and incident CVD events (Table 3). Longer LTL was associated with lower risk of incident ischemic stroke (hazard ratio [HR] 0.69, 95% CI 0.48, 0.99, $p=0.044$) and all-cause mortality (HR 0.81, 95% CI 0.67, 0.97, $p=0.026$) in age and sex adjusted models. These associations were attenuated and no longer significant in models fully adjusted for additional CVD risk factors. As a supplemental analysis, we also assessed associations of LTL with incident events using Exam 2 and Exam 3 respectively as a baseline, to account for changes in CVD risk factor profile during follow-up. No significant associations were observed (data not shown), due either to the impact of the incident risk factors (such as additional individuals developing diabetes or high cholesterol) or lack of power due to the smaller number of events occurring after Exam 2 or 3 compared to Exam 1.

DISCUSSION

In this study of LTL in a population-based cohort of 2,518 AA men and women, there were three important findings. First, no demographic or CVD risk factors, apart from age, sex, and smoking, were associated with LTL. Second, while we observed modest associations of LTL with lower odds of presence of CAC and higher ABI, indicative of decreased risk of subclinical atherosclerosis and peripheral arterial disease, most subclinical CVD measures

assessed were not associated with LTL. Third, we found nominal associations of higher LTL with decreased risk of mortality, but these associations became non-significant when adjusted for common CVD risk factors. In summary, we observed little evidence in this population that LTL is a clinically useful predictor of CVD risk.

In our analysis of demographic and CVD risk factors, we replicated the compelling association of LTL with age and sex, which we adjusted for in all models, as well as a more modest association between smoking and shorter LTL, as has been previously observed [15]. Interestingly, we did not replicate previous associations with measures such as socioeconomic status [17] (as assessed by income and education) and BMI [18] in JHS when adjusting for age and sex.

In our analysis of LTL with subclinical CVD measures, modest association of increased LTL with lower odds of presence of CAC was observed in models adjusted for traditional CVD risk factors; however, no association with CAC analyzed as a continuous measure remained in these fully adjusted models. Inverse association of LTL with CAC has been observed previously [9, 10, 38], but this association has been reported to be stronger in EA vs AA populations and is not always significant in AA participants alone [9, 10]. We also found that the top quartile of LTL was associated with higher ABI in JHS. Few studies have examined the association of ABI with LTL, but our findings are broadly concordant with two small studies from China. In the first study, shorter LTL and was associated with higher risk of peripheral arterial disease (defined as ABI ≤ 0.9 or symptoms of peripheral arterial disease) [39], and in the second study an inverse association was observed between LTL and rate of decline in ABI in women only [40].

In models adjusted for age and sex, analyzing LTL as either a continuous or discrete quartile measure, we did not observe associations of LTL with IMT, AAC, LVM, or LVMI. The previous literature shows inconsistent association of LTL with these subclinical CVD measures, with limited data available in large cohorts, particularly for AAC. In cross-sectional analyses, several European cohorts showed no association of longer LTL with IMT when adjusting for conventional CVD risk factors [41–43], or found associations in only a subset of participants (for example obese males) [44]. However, in a Native American population from the Strong Heart Family Study shorter baseline LTL predicted incidence and progression of carotid plaque [11]. Some previous studies in European populations have found significant positive associations between LVM and LTL, both in cross-sectional analyses [13] and with longitudinal assessment of LVM (median 7.4 years after LTL measurement) [12], but others have found no association [41, 45].

In age- and sex-adjusted models we observed a modest association of longer LTL with decreased mortality and ischemic stroke risk in AAs, but no association was observed with incident heart failure hospitalizations or CHD. Association of lower mortality risk and ischemic stroke with longer LTL was no longer significant in JHS participants after adjustment for established CVD risk factors, suggesting the possibility that the association with these outcomes may be mediated through the association of LTL with CVD risk factors. Nominally significant association of longer LTL with decreased risk of mortality in JHS is supported by some studies including AA participants, including the Cardiovascular Health

Study [2] and the National Health and Nutrition Examination Survey [46], but not by the Women's Health Initiative (WHI) [25] or the Health, Aging, and Body Composition study [47]. In fact, in the WHI, there was a suggestion that *shorter* LTL may be associated with decreased mortality in AA women, possibly attributable to cancer deaths [25]. Two recent meta-analyses, including predominantly participants of European and East Asian heritage, found a significantly increased risk of CHD and stroke with shorter LTL [4, 5]. However, a recent prospective study in a Danish cohort with long-term follow-up (29 years) found no associations with all CVD or stroke and LTL, though a nominal inverse association with ischemic heart disease was observed [48]. Existing data on heart failure and LTL is limited; case-control analyses in predominantly EA participants have also found an association of heart failure with shorter LTL, particularly in those with other coincident CVD [49].

Several factors may explain differences between our findings and previously published work. It should be noted that few available studies have specifically assessed AA populations, and heterogeneity between EA and AA populations has been observed in some studies. For example, in the WHI, associations between shorter LTL and increased CHD events were observed in EA, but not AA, participants [25]. These WHI results suggest that differences in CVD risk profile between AAs and other population groups, or the longer LTL among AAs compared to EAs, may explain the lack of association of LTL with some traits (notably CHD events) in the JHS. The JHS also did not include any EA participants, so we cannot directly assess significant differences between African and European ancestry populations in the association of LTL with CVD risk. Our power to detect associations of LTL with incident CVD events and mortality may be limited, given previous estimates of effect size after adjustment for CVD risk factors. For example, in a large population based study from Copenhagen, shorter LTL was associated with an estimated 6–10% increase in risk of MI, CHD, and death per 1 kb decrease in LTL when models were adjusted for established CVD risk factors [3]. Heterogeneity in methods of telomere length assessment may also contribute to the conflicting literature concerning LTL; many existing studies use quantitative polymerase chain reaction (qPCR) methods as opposed to the Southern blot method utilized here (for example [9, 46]). The correlation between these methods displays significant deviation from linearity, and southern blots have lower inter-assay variation than qPCR methods [50]. In a comparison of Southern blot and qPCR methods in the Health Aging and Body Composition (Health ABC) study, LTL was modestly correlated between methods ($r^2=0.27$), and only the Southern blot method measure demonstrated the known association of longer LTL with African as compared to European ancestry [51]. Differences in methods, as opposed to true differences in the relationship between LTL and CVD, may thus explain some of the observed variation between studies.

Strengths of the study include the large sample of both male and female AA participants, an understudied group for LTL at high risk of CVD, and availability of both incident event and subclinical measures for assessment of CVD risk. For many analyzed traits, few if any studies of associations with LTL were previously available in an AA population. Limitations include a single time-point for subclinical CVD measures and a relatively limited number of events during follow-up, particularly for stroke subtypes, reducing power to detect modest associations with LTL. We also did not have data to assess incident diagnoses other than CVD (such as cancer) that may also be associated with telomere length [52]. The JHS also

recruited AA participants from a single geographic region (Jackson, MS), potentially reducing many sources of heterogeneity common in multi-center studies but limiting generalizability of results to other regions of the U.S. Moreover, while clinical variables and LTL were from JHS Exam 1, assessment of CAC and AAC was performed at JHS Exam 2. Heart failure was also not assessed at baseline, so incident heart failure is assessed only from 2005–2012. Finally, we only examined associations with a cross-sectional measure of LTL, not change in telomere length over time, which in some studies has been more strongly associated with subclinical CVD measures [41]. More studies, particularly in African Americans, are also needed to assess whether CVD risk factors, such as hypertension, hyperglycemia, and obesity, are associated with differing rates of telomere attrition [53–55].

A large and sometimes conflicting literature has examined LTL as a potential biomarker for CVD and other aging-related disease states. Among a community-based cohort of AAs, longer LTL was nominally associated with decreased odds of presence of CAC and increased ABI, indicative of decreased risk of subclinical atherosclerosis and peripheral arterial disease, but there were no significant associations with other CVD indices or mortality after adjustment for established risk factors. These findings do not offer strong support for LTL as an independent or clinically useful biomarker of CVD risk in AAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FINANCIAL SUPPORT

This research was supported by R01 HL116446 (Wilson/Aviv/Reiner). Dr. Raffield is supported by T32 HL129982 from the National Heart, Lung, and Blood Institute, and Dr. Wilson is supported by U54GM115428 from the National Institute of General Medical Sciences. The Jackson Heart Study is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services. The funders had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, and in the preparation, review, or approval of the manuscript.

We thank the Jackson Heart Study (JHS) participants and staff for their contributions to this work.

References

1. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci*. 2011; 66(2):202–13. DOI: 10.1093/gerona/glq180 [PubMed: 21030466]
2. Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2011; 66(4):421–9. DOI: 10.1093/gerona/glq224 [PubMed: 21289018]
3. Weischer M, Bojesen SE, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, et al. Short telomere length, myocardial infarction, ischemic heart disease, and early death. *Arterioscler Thromb Vasc Biol*. 2012; 32(3):822–9. DOI: 10.1161/atvbaha.111.237271 [PubMed: 22199369]

4. D'Mello MJ, Ross SA, Briel M, Anand SS, Gerstein H, et al. Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. *Circ Cardiovasc Genet.* 2015; 8(1):82–90. DOI: 10.1161/circgenetics.113.000485 [PubMed: 25406241]
5. Haycock PC, Heydon EE, Kaptoge S, Butterworth AS, Thompson A, et al. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ.* 2014; 349:g4227. [PubMed: 25006006]
6. Benetos AKJ, Susser E, Kimura M, Sinnreich R, Chen W, Steenstrup T, Christensen K, Herbig U, von Bornemann Hjelmberg J, Srinivasan SR, Berenson GS, Labat C, Aviv A. Tracking and fixed ranking of leukocyte telomere length across the adult life course. *Aging cell.* 2013; 12:615–621. [PubMed: 23601089]
7. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet.* 2013; 45(4):422–7. 427e1–2. DOI: 10.1038/ng.2528 [PubMed: 23535734]
8. Scheller Madrid A, Rode L, Nordestgaard BG, Bojesen SE. Short Telomere Length and Ischemic Heart Disease: Observational and Genetic Studies in 290 022 Individuals. *Clin Chem.* 2016; 62(8): 1140–9. DOI: 10.1373/clinchem.2016.258566 [PubMed: 27259814]
9. Mainous AG 3rd, Codd V, Diaz VA, Schoepf UJ, Everett CJ, et al. Leukocyte telomere length and coronary artery calcification. *Atherosclerosis.* 2010; 210(1):262–7. DOI: 10.1016/j.atherosclerosis.2009.10.047 [PubMed: 19945703]
10. Hunt SC, Kimura M, Hopkins PN, Carr JJ, Heiss G, et al. Leukocyte telomere length and coronary artery calcium. *Am J Cardiol.* 2015; 116(2):214–8. DOI: 10.1016/j.amjcard.2015.03.060 [PubMed: 25960381]
11. Chen S, Lin J, Matsuguchi T, Blackburn E, Yeh F, et al. Short leukocyte telomere length predicts incidence and progression of carotid atherosclerosis in American Indians: the Strong Heart Family Study. *Aging (Albany NY).* 2014; 6(5):414–27. DOI: 10.18632/aging.100671 [PubMed: 24902894]
12. Kuznetsova T, Codd V, Brouillette S, Thijs L, Gonzalez A, et al. Association between left ventricular mass and telomere length in a population study. *Am J Epidemiol.* 2010; 172(4):440–50. DOI: 10.1093/aje/kwq142 [PubMed: 20660518]
13. Vasan RS, Demissie S, Kimura M, Cupples LA, White C, et al. Association of leukocyte telomere length with echocardiographic left ventricular mass: the Framingham heart study. *Circulation.* 2009; 120(13):1195–202. DOI: 10.1161/circulationaha.109.853895 [PubMed: 19752323]
14. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet.* 2005; 366(9486):662–4. DOI: 10.1016/s0140-6736(05)66630-5 [PubMed: 16112303]
15. Shamas MA. Telomeres, lifestyle, cancer, and aging. *Curr Opin Clin Nutr Metab Care.* 2011; 14(1):28–34. DOI: 10.1097/MCO.0b013e32834121b1 [PubMed: 21102320]
16. Eisenberg DT, Hayes MG, Kuzawa CW. Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. *Proc Natl Acad Sci U S A.* 2012; 109(26):10251–6. DOI: 10.1073/pnas.1202092109 [PubMed: 22689985]
17. Cherkas LF, Aviv A, Valdes AM, Hunkin JL, Gardner JP, et al. The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell.* 2006; 5(5):361–5. DOI: 10.1111/j.1474-9726.2006.00222.x [PubMed: 16856882]
18. Muezzinler A, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. *Obes Rev.* 2014; 15(3):192–201. DOI: 10.1111/obr.12126 [PubMed: 24165286]
19. Andrew T, Aviv A, Falchi M, Surdulescu GL, Gardner JP, et al. Mapping genetic loci that determine leukocyte telomere length in a large sample of unselected female sibling pairs. *Am J Hum Genet.* 2006; 78(3):480–6. DOI: 10.1086/500052 [PubMed: 16400618]
20. Vasa-Nicotera M, Brouillette S, Mangino M, Thompson JR, Braund P, et al. Mapping of a major locus that determines telomere length in humans. *Am J Hum Genet.* 2005; 76(1):147–51. DOI: 10.1086/426734 [PubMed: 15520935]

21. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 2015; 131(4):e29–322. DOI: 10.1161/cir.000000000000152 [PubMed: 25520374]
22. Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, et al. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. *Aging Cell*. 2008; 7(4):451–8. DOI: 10.1111/j.1474-9726.2008.00397.x [PubMed: 18462274]
23. Bild DE, Detrano R, Peterson D, Guerci A, Liu K, et al. Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*. 2005; 111(10): 1313–20. DOI: 10.1161/01.cir.0000157730.94423.4b [PubMed: 15769774]
24. Gardin JM, Wagenknecht LE, Anton-Culver H, Flack J, Gidding S, et al. Relationship of cardiovascular risk factors to echocardiographic left ventricular mass in healthy young black and white adult men and women. The CARDIA study. *Coronary Artery Risk Development in Young Adults*. *Circulation*. 1995; 92(3):380–7. [PubMed: 7634452]
25. Carty CL, Kooperberg C, Liu J, Herndon M, Assimes T, et al. Leukocyte Telomere Length and Risks of Incident Coronary Heart Disease and Mortality in a Racially Diverse Population of Postmenopausal Women. *Arterioscler Thromb Vasc Biol*. 2015; 35(10):2225–31. DOI: 10.1161/atvbaha.115.305838 [PubMed: 26249011]
26. Taylor HA Jr, Wilson JG, Jones DW, Sarpong DF, Srinivasan A, et al. Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study. *Ethn Dis*. 2005; 15(4 Suppl 6):S6–4–17.
27. Carpenter MA, Crow R, Steffes M, Rock W, Heilbraun J, et al. Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study. *Am J Med Sci*. 2004; 328(3):131–44. [PubMed: 15367870]
28. Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, et al. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nat Protoc*. 2010; 5(9):1596–607. DOI: 10.1038/nprot.2010.124 [PubMed: 21085125]
29. Wang W, Young BA, Fulop T, de Boer IH, Boulware LE, et al. Effects of serum creatinine calibration on estimated renal function in african americans: the Jackson heart study. *Am J Med Sci*. 2015; 349(5):379–84. DOI: 10.1097/maj.0000000000000446 [PubMed: 25806862]
30. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*. 2010; 121(4):586–613. DOI: 10.1161/circulationaha.109.192703 [PubMed: 20089546]
31. Liu J, Fox CS, Hickson D, Sarpong D, Ekunwe L, et al. Pericardial adipose tissue, atherosclerosis, and cardiovascular disease risk factors: the Jackson heart study. *Diabetes care*. 2010; 33(7):1635–9. DOI: 10.2337/dc10-0245 [PubMed: 20413524]
32. Deere B, Griswold M, Lirette S, Fox E, Sims M. Life Course Socioeconomic Position and Subclinical Disease: The Jackson Heart Study. *Ethn Dis*. 2016; 26(3):355–62. DOI: 10.18865/ed.26.3.355 [PubMed: 27440975]
33. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986; 57(6):450–8. [PubMed: 2936235]
34. Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, et al. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation*. 2012; 126(24):2890–909. DOI: 10.1161/CIR.0b013e318276fbc [PubMed: 23159553]
35. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology*. 2005; 234(1):35–43. DOI: 10.1148/radiol.2341040439 [PubMed: 15618373]
36. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990; 15(4): 827–32. [PubMed: 2407762]

37. Keku E, Rosamond W, Taylor HA Jr, Garrison R, Wyatt SB, et al. Cardiovascular disease event classification in the Jackson Heart Study: methods and procedures. *Ethn Dis.* 2005; 15(4 Suppl 6):S6-62–70.
38. Kark JD, Nassar H, Shaham D, Sinnreich R, Goldberger N, et al. Leukocyte telomere length and coronary artery calcification in Palestinians. *Atherosclerosis.* 2013; 229(2):363–8. DOI: 10.1016/j.atherosclerosis.2013.05.030 [PubMed: 23880188]
39. Zhang W, Chen Y, Yang X, Fan J, Mi X, et al. Functional haplotypes of the hTERT gene, leukocyte telomere length shortening, and the risk of peripheral arterial disease. *PLoS ONE.* 2012; 7(10):e47029.doi: 10.1371/journal.pone.0047029 [PubMed: 23082138]
40. Woo J, Tang NL, Suen E, Leung J. Shorter telomere length is associated with greater decrease in ankle-brachial index in elderly Chinese women but not men. *Angiology.* 2011; 62(1):87–91. DOI: 10.1177/0003319710371618 [PubMed: 20504835]
41. Masi S, D'Aiuto F, Martin-Ruiz C, Kahn T, Wong A, et al. Rate of telomere shortening and cardiovascular damage: a longitudinal study in the 1946 British Birth Cohort. *Eur Heart J.* 2014; 35(46):3296–303. DOI: 10.1093/eurheartj/ehu226 [PubMed: 24957070]
42. De Meyer T, Rietzschel ER, De Buyzere ML, Langlois MR, De Bacquer D, et al. Systemic telomere length and preclinical atherosclerosis: the Asklepios Study. *Eur Heart J.* 2009; 30(24):3074–81. DOI: 10.1093/eurheartj/ehp324 [PubMed: 19687155]
43. Fernandez-Alvira JM, Fuster V, Dorado B, Soberon N, Flores I, et al. Short Telomere Load, Telomere Length, and Subclinical Atherosclerosis: The PESA Study. *J Am Coll Cardiol.* 2016; 67(21):2467–76. DOI: 10.1016/j.jacc.2016.03.530 [PubMed: 27230041]
44. O'Donnell CJ, Demissie S, Kimura M, Levy D, Gardner JP, et al. Leukocyte telomere length and carotid artery intimal medial thickness: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol.* 2008; 28(6):1165–71. DOI: 10.1161/atvbaha.107.154849 [PubMed: 18388332]
45. Denil SL, Rietzschel ER, De Buyzere ML, Van Daele CM, Segers P, et al. On cross-sectional associations of leukocyte telomere length with cardiac systolic, diastolic and vascular function: the Asklepios study. *PLoS ONE.* 2014; 9(12):e115071.doi: 10.1371/journal.pone.0115071 [PubMed: 25506937]
46. Needham BL, Rehkopf D, Adler N, Gregorich S, Lin J, et al. Leukocyte telomere length and mortality in the National Health and Nutrition Examination Survey, 1999–2002. *Epidemiology.* 2015; 26(4):528–35. DOI: 10.1097/ede.0000000000000299 [PubMed: 26039272]
47. Njajou OT, Hsueh WC, Blackburn EH, Newman AB, Wu SH, et al. Association between telomere length, specific causes of death, and years of healthy life in health, aging, and body composition, a population-based cohort study. *J Gerontol A Biol Sci Med Sci.* 2009; 64(8):860–4. DOI: 10.1093/gerona/glp061 [PubMed: 19435951]
48. Ellehoj H, Bendix L, Osler M. Leucocyte Telomere Length and Risk of Cardiovascular Disease in a Cohort of 1,397 Danish Men and Women. *Cardiology.* 2016; 133(3):173–7. DOI: 10.1159/000441819 [PubMed: 26666879]
49. van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J Am Coll Cardiol.* 2007; 49(13):1459–64. DOI: 10.1016/j.jacc.2007.01.027 [PubMed: 17397675]
50. Aviv A, Hunt SC, Lin J, Cao X, Kimura M, et al. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Res.* 2011; 39(20):e134.doi: 10.1093/nar/gkr634 [PubMed: 21824912]
51. Elbers CC, Garcia ME, Kimura M, Cummings SR, Nalls MA, et al. Comparison between southern blots and qPCR analysis of leukocyte telomere length in the health ABC study. *J Gerontol A Biol Sci Med Sci.* 2014; 69(5):527–31. DOI: 10.1093/gerona/glt121 [PubMed: 23946336]
52. Stone RC, Horvath K, Kark JD, Susser E, Tishkoff SA, et al. Telomere Length and the Cancer-Atherosclerosis Trade-Off. *PLoS Genet.* 2016; 12(7):e1006144.doi: 10.1371/journal.pgen.1006144 [PubMed: 27386863]
53. Revesz D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BW. Longitudinal Associations Between Metabolic Syndrome Components and Telomere Shortening. *J Clin Endocrinol Metab.* 2015; 100(8):3050–9. DOI: 10.1210/jc.2015-1995 [PubMed: 26133009]

54. Weischer M, Bojesen SE, Nordestgaard BG. Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. *PLoS Genet.* 2014; 10(3):e1004191.doi: 10.1371/journal.pgen.1004191 [PubMed: 24625632]
55. Huzen J, Wong LS, van Veldhuisen DJ, Samani NJ, Zwinderman AH, et al. Telomere length loss due to smoking and metabolic traits. *J Intern Med.* 2014; 275(2):155–63. DOI: 10.1111/joim.12149 [PubMed: 24118582]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

- Telomere shortening has been linked to aging and cardiovascular disease (CVD).
- We assessed associations of telomere length with CVD in the Jackson Heart Study.
- We observed modest associations with subclinical CVD measures.
- However, no independent associations were observed for incident CVD or mortality.
- Further investigation is needed in large African American populations.

Table 1

Participant characteristics at baseline (Exam 1) and association of these characteristics with quartiles of leukocyte telomere length (LTL), adjusted for age and sex.

Characteristics	Mean (SD)/Count (%) In Q1	Mean (SD)/Count (%) in Q2	Mean (SD)/Count (%) in Q3	Mean (SD)/Count (%) in Q4	p-value for trend across quartiles, adjusted for age and sex
N	629	630	630	629	
LTL	6.33 (0.30)	6.93 (0.12)	7.38 (0.15)	8.08 (0.36)	
Age, years	61.32 (11.06)	56.81 (11.77)	53.80 (11.64)	49.09 (11.82)	<0.001
Female	342 (54%)	380 (60%)	388 (62%)	434 (69%)	<0.001
BMI, Kg/m ²	31.67 (7.23)	32.15 (7.44)	31.92 (7.36)	32.49 (7.67)	0.300
Current smoker	88 (14%)	96 (15%)	103 (16%)	72 (12%)	0.014
Diabetes	184 (29%)	140 (22%)	126 (20%)	111 (18%)	0.204
Systolic BP (mmHg)	130.16 (17.80)	127.83 (16.79)	126.85 (16.41)	124.87 (15.81)	0.772
Diastolic BP (mmHg)	75.08 (9.10)	75.57 (8.20)	76.48 (9.06)	76.38 (8.84)	0.151
Statin therapy	90 (16%)	70 (12%)	70 (12%)	44 (7%)	0.620
Fasting HDL (mg/dL)	50.92 (14.61)	50.95 (14.71)	50.74 (14.59)	51.34 (14.41)	0.920
Fasting LDL (mg/dL)	125.33 (37.12)	126.45 (35.41)	124.70 (39.41)	126.49 (36.42)	0.343
Fasting TRI (mg/dL)	112.68 (77.06)	115.30 (131.73)	113.07 (106.13)	105.90 (75.30)	0.652
Fasting total cholesterol (mg/dL)	199.06 (40.43)	199.31 (38.11)	198.03 (43.07)	198.95 (40.61)	0.248
Subclinical disease					
CAC *	227.76 (475.09)	167.89 (496.19)	146.12 (402.14)	103.98 (397.08)	0.996
CAC categories *					
= 0	121 (36%)	180 (49%)	201 (56%)	223 (64%)	0.237
>0	214 (64%)	191 (51%)	161 (44%)	125 (36%)	
AAC *	1141.95 (1605.42)	1070.10 (1941.42)	775.63 (1519.71)	559.96 (1216.78)	0.247
AAC categories *					
=0	71 (21%)	124 (33%)	122 (34%)	159 (46%)	0.105
>0	263 (79%)	247 (67%)	240 (66%)	189 (54%)	
ABI	1.10 (0.17)	1.12 (0.15)	1.12 (0.14)	1.15 (0.13)	0.082

Characteristics	Mean (SD)/Count (%) In Q1	Mean (SD)/Count (%) in Q2	Mean (SD)/Count (%) in Q3	Mean (SD)/Count (%) in Q4	p-value for trend across quartiles, adjusted for age and sex
ABI categories					
0.9 ABI 1.4	460 (89%)	500 (92%)	508 (93%)	514 (96%)	0.151
0 ABI < 0.9	56 (11%)	42 (8%)	41 (7%)	19 (4%)	
cIMT	0.85 (0.26)	0.81 (0.24)	0.77 (0.20)	0.72 (0.21)	0.820
LV mass, g	158.99 (45.34)	152.07 (41.45)	149.12 (38.73)	144.06 (37.03)	0.944
LV mass index	38.20 (10.95)	36.33 (9.59)	35.70 (9.09)	35.28 (9.12)	0.489
LV hypertrophy	45 (13%)	32 (8%)	23 (6%)	24 (6%)	0.233

Values are mean (SD) for continuous variables or n (%) for categorical variables. A natural log transformation was used for TRI to assess LTL associations.

* CAC and *AAC measures were not available at Exam 1; these measures are from Jackson Heart Study Exam 2 (2005–2008).

LTL, leukocyte telomere length; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRI, triglycerides; CAC, coronary artery calcification; AAC, abdominal aorta calcification; ABI, ankle-brachial index; CIMT, carotid intimal-medial thickness; LV, left ventricular mass; SD, standard deviation

Table 2

Associations of leukocyte telomere length (LTL) with subclinical cardiovascular disease (CVD) measures in the Jackson Heart Study cohort.

Measure	Model 1 ^a		Model 2 ^b		Model 3 ^c	
	Difference/OR (95% CI)	p-value	Difference/OR (95% CI)	p-value	Difference/OR (95% CI)	p-value
Presence of CAC	0.794 (0.655, 0.964)	0.020	0.837 (0.686, 1.020)	0.078	0.810 (0.656, 0.9998)	0.0498
CAC (continuous)	-0.535 (-1.066, -0.003)	0.049	-0.379 (-0.908, 0.150)	0.160	-0.403 (-0.945, 0.140)	0.146
Presence of AAC	0.884 (0.719, 1.086)	0.239	0.943 (0.762, 1.167)	0.591	0.918 (0.733, 1.149)	0.453
AAC (continuous)	-0.286 (-0.763, 0.191)	0.240	-0.097 (-0.557, 0.363)	0.680	-0.107 (-0.576, 0.361)	0.653
ABI	0.007 (-0.002, 0.017)	0.136	0.007 (-0.003, 0.017)	0.157	0.008 (-0.002, 0.018)	0.109
cIMT	-0.013 (-0.034, 0.007)	0.201	-0.011 (-0.032, 0.010)	0.297	-0.013 (-0.034, 0.008)	0.227
LV mass	-0.012 (-0.039, 0.015)	0.376	0.011 (-0.014, 0.036)	0.398	0.009 (-0.017, 0.034)	0.496
LV mass index	-0.010 (-0.038, 0.017)	0.453	0.013 (-0.013, 0.038)	0.334	0.009 (-0.017, 0.035)	0.489

^aModel 1: Adjusted for age and sex.

^bModel 2: Adjusted for Model 1 + body mass index (BMI), current smoking.

^cModel 3: Adjusted for Model 2 + systolic blood pressure (SBP), diabetes, and low density lipoprotein (LDL) cholesterol.

Presence of CAC and AAC defined as Agatston score > 0. Relationships between LTL and subclinical CVD measures are assessed using linear or logistic regression models. Tobit regression models are used for log transformed AAC and CAC. cIMT, LV mass, and LV mass index are also log transformed. Difference in a continuous outcome or odds ratio for presence of CAC (or AAC) is reported for each 1 kb increment of LTL.

Abbreviations as in Table 1.

Table 3

Associations of leukocyte telomere length with incident events and all-cause mortality (through 2012) in the Jackson Heart Study cohort.

Outcome	Cases	N	Model 1 ^b			Model 2 ^c			Model 3 ^d		
			HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Stroke (all)	84	2,365	0.71	(0.50, 1.00)	0.052	0.74	(0.52, 1.04)	0.083	0.73	(0.50, 1.06)	0.093
Stroke subtypes											
Ischemic	76	2,365	0.69	(0.48, 0.99)	0.044	0.72	(0.50, 1.03)	0.076	0.70	(0.47, 1.04)	0.079
Hemorrhagic	7	2,365	1.02	(0.31, 3.32)	0.979	1.01	(0.31, 3.31)	0.993	1.04	(0.32, 3.35)	0.954
CHD	107	2,283	1.02	(0.75, 1.39)	0.891	1.04	(0.77, 1.42)	0.783	1.19	(0.85, 1.67)	0.318
Hard CHD ^a	79	2,290	1.03	(0.72, 1.47)	0.873	1.04	(0.73, 1.49)	0.820	1.28	(0.85, 1.91)	0.235
Heart failure	123	2,168	0.85	(0.63, 1.13)	0.270	0.88	(0.66, 1.17)	0.367	0.99	(0.73, 1.34)	0.959
Mortality	285	2,518	0.81	(0.67, 0.97)	0.026	0.86	(0.71, 1.04)	0.119	0.91	(0.74, 1.12)	0.377

^aHard CHD is defined as myocardial infarction and fatal CHD.

CHD, coronary heart disease; HR, hazard ratio; CI, confidence interval.

^bModel 1: Adjusted for age and sex.

^cModel 2: Adjusted for Model 1 + body mass index (BMI), current smoking.

^dModel 3: Adjusted for Model 2 + systolic blood pressure (SBP), diabetes, and low density lipoprotein (LDL) cholesterol.