Effect of Healthy Lifestyle Behaviors on the Association Between Leukocyte Telomere Length and Coronary Artery Calcium

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The telomere length is an indicator of biologic aging, and shorter telomeres have been associated with coronary artery calcium (CAC), a validated indicator of coronary atherosclerosis. It is unclear, however, whether healthy lifestyle behaviors affect the relation between telomere length and CAC. In a sample of subjects aged 40 to 64 years with no previous diagnosis of coronary heart disease, stroke, diabetes mellitus, or cancer (n = 318), healthy lifestyle behaviors of greater fruit and vegetable consumption, lower meat consumption, exercise, being at a healthy weight, and the presence of social support were examined to determine whether they attenuated the association between a shorter telomere length and the presence of CAC. Logistic regression analyses controlling for age, gender, race/ethnicity, and Framingham risk score revealed that the relation between having shorter telomeres and the presence of CAC was attenuated in the presence of high social support, low meat consumption, and high fruit and vegetable consumption. Those with shorter telomeres and these characteristics were not significantly different from those with longer telomeres. Conversely, the subjects with shorter telomeres and less healthy lifestyles had a significantly increased risk of the presence of CAC: low fruit and vegetable consumption (odds ratio 3.30, 95% confidence interval 1.61 to 6.75), high meat consumption (odds ratio 3.33, 95% confidence interval 1.54 to 7.20), and low social support (odds ratio 2.58, 95% confidence interval 1.24 to 5.37). Stratification by gender yielded similar results for men; however, among women, only fruit and vegetable consumption attenuated the shorter telomere length and CAC relation. In conclusion, the results of the present study suggest that being involved in healthy lifestyle behaviors might attenuate the association between shorter telomere length and coronary atherosclerosis, as identified using CAC.
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A shorter telomere length has been associated with increased coronary artery calcium (CAC).[1] A shorter telomere length has also been associated with a number of risk factors for coronary heart disease, such as a sedentary lifestyle, high body mass index, and life stress.[2,3] Additionally, nutrition has been shown to affect the telomere length.[4–6] Thus, the telomere length has been associated with both modifiable risk factors for coronary heart disease, such as weight and nutrition, and CAC. It is unclear how lifestyle factors affect the relation between telomere length and CAC. Because a shorter telomere length and sedentary lifestyle have been independently associated with having CAC, one could assume that those with longer telomeres who exercise would be less likely to have evidence of CAC than those with shorter telomeres who do not exercise. However, it is unclear whether performing a healthy lifestyle behavior does attenuate the relation between telomere length and CAC in this manner. The present study examined the relation between telomere length and CAC in the context of the lifestyle characteristics associated with coronary heart disease risk. Specifically, we evaluated the effect of healthy lifestyle characteristics on telemore length and the presence of CAC.

Methods

Participants were recruited through health fairs, flyers, and advertisements posted at the local Health Science University. A of 318 subjects, 40 to 64 years old and free of diagnosed diabetes, coronary heart disease, stroke, and cancer were studied. The subjects were 57% non-Hispanic whites, 41% non-Hispanic blacks, and 2% of other races/ethnicities. These proportions were consistent with the racial/ethnic demographics of the area.

The leukocyte telomere length was measured using a quantitative polymerase chain reaction (PCR)-based technique that compares the telomere repeat sequence copy number to the single-copy gene (36b4) copy number in a given sample.[7] Duplicate DNA samples (isolated using the Gentra Puregene Blood Kit, Qiagen, Hilden, Germany) were amplified in parallel 25-μL PCR reactions composed of 15-ng genomic DNA, 1× SensiMix NoRef Sybr Green master mix, 1× Sybr Green (Quantace, United Kingdom)

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Table 1
Demographics

Variable	Total Sample (n = 318)	Men (n = 144)	Women (n = 174)	p Value*
Mean age (years)	51.3	51.2	51.4	0.73
Race/ethnicity				0.14
Non-Hispanic white	57%	64%	51%	
Non-Hispanic black	41%	35%	46%	
Hispanic	1%	1%	1%	
Other	1%	1%	2%	
Coronary artery calcium				<0.01
0	69%	59%	78%	
>0	31%	41%	22%	
Telomere length (telomere/single-copy gene ratio)				0.03
≤1.521 (short)	33%	39%	28%	
>1.521 (long)	67%	61%	72%	
Framingham risk score (10-year risk)				<0.01
<10% (low)	86%	71%	99%	
≥10% (high)	14%	29%	1%	
Fruit and vegetable consumption (servings/d)				0.02
≤2.5 (low)	50%	57%	44%	
>2.5 (high)	50%	43%	56%	
Meat consumption (servings/d)				0.94
≤0.4286 (low)	54%	54%	55%	
>0.4286 (high)	46%	46%	45%	
Exercise				<0.05
Sometimes or less (low)	52%	46%	57%	
Often or very often (high)	48%	54%	43%	
Body mass index (kg/m^2)				0.82
≤28.1 (low)	50%	51%	49%	
>28.1 (high)	50%	49%	51%	
Social support (scaled)				<0.01
≤6.75 (low)	54%	65%	44%	
>6.75 (high)	46%	35%	56%	

* p <0.05 indicate significant difference between men and women.

and either 300 nmol/L of telomere-specific primers (forward: 5′-GGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; reverse: 5′- GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′) or 300 nmol/L of the 36B4 forward primer (5′-CAGCAAGTGGGAAGGTGTAATCC-3′) primer and 500 nM of the 36B4 reverse primer (5′-CCCATTCTATCATCAACGGGTACAA-3′). All PCRs were run on a Corbett Research Rotor-Gene 6000 Real-Time Thermal Cycler (Corbett Research, Cambridge, United Kingdom). The thermal cycling profile begins with a 95°C incubation for 10 minutes to activate the Taq DNA polymerase, followed by cycling of 15 seconds at 95°C and 1 minute at 58°C for either 20 cycles (telomere) or 30 cycles (36B4). Before running the samples, the linear range of the assay was determined by generating a standard curve using serially diluted DNA (200 to 1.56 ng in twofold dilutions) in quadruplicate. Both PCR reactions exhibited good linearity across this input range (r^2 >0.99). The test samples were checked to confirm that they decreased within this range and any that did not were diluted as necessary and rerun. The specificity of all amplifications was determined using a melting curve analysis. A total of 48 study samples, 1 calibrator sample, and 1 no-template control sample (all in duplicate) were processed per run.

The PCR data were analyzed using the comparative quantitation approach, as previously described, and implemented with the Corbett Research Rotor-Gene 6000, version 1.7 (Corbett Research), analysis software.[8] During this analysis, the amplification efficiency was calculated for each sample, along with the mean efficiency of the run, which was used in calculating the relative concentration of each sample relative to the calibrator sample. This calculation, coupled with using the same calibrator samples on all runs, allowed for any inter-run variation. This process was done for both telomere (T) and single-copy (S) gene reactions and for the telomere length, expressed as a ratio of the 2 (T/S ratio), of the mean data from the duplicate runs. All analyses were done without knowing the patient characteristics.

The telomere length assay (T/S ratio) was checked for reproducibility by rerunning 76 samples on a different day. The correlation *(r)* between the 2 runs was 0.946. The first tertile of the telomere length was a T/S ratio of ≤1.521, the second tertile, a T/S ratio of 1.522 to 1.776, and the third tertile, a T/S ratio of >1.776. The minimum T/S ratio was 1.025, and the maximum T/S ratio was 2.623. The telomere length was normally distributed. For our analyses, the first tertile was compared to the combined second and third tertiles.

Several lifestyle variables that might act as buffers to the relation between telomere length and CAC were measured. Each variable was split at the median and combined with the T/S ratio to create a 4-part variable, indicating 2 positive

characteristics, 2 negative characteristics, and mixed positive and negative characteristics.

The variables assessed included fruit and vegetable consumption (median 2.50 servings/d) and meat consumption (median 0.4286 servings/d). To maximize the accuracy while assessing this variable, participants had available to them written information and examples regarding the size of a portion that constitutes a serving for different foods within these food groups. For instance, participants were told that 1 serving of vegetables would be 1/2 cup of chopped raw or cooked vegetables or 1 cup of leafy raw vegetables and that a ½ baseball would be equivalent to ½ cup and a fist would be equivalent to 1 cup. For fruit, 1 serving was described as equivalent to ½ cup canned or 1 medium fruit and that a tennis ball was equivalent to ½ cup of canned fruit. For meat, 1 serving was described as 2.5 to 3 oz of cooked lean meat, with the palm, not including the fingers or thumb, an example of a 3-oz serving. In addition, plastic visual aids were available that represented this portion size information. Exercise was characterized as never, seldom, or sometimes versus often or very often, and the body mass index (median 28.1 kg/m^2) was calculated using the height and weight measurements collected through physical examination.

Social support was determined using the 12 items of the Multidimensional Scale of Perceived Social Support, which uses a 7-point rating scale ranging from very strongly disagree (score 1) to very strongly agree (score 7).[9] The 12 questions used in this scale identify the degree of help, comfort, and support available to subjects from family, friends, or a special person in different circumstances, such as when they are in need, want to share emotions, or have problems. The average score on this scale was computed for each participant (median 6.75) and was included in the analysis because of its role as a possible attenuator of life stress.[10]

CAC scoring was performed using a dual-source computed tomography scanner (Somatom Definition, Siemens Medical Solutions, Malvern, Pennsylvania) using prospective electrocardiographic triggering and a reconstructed section thickness of 1.5 mm without the use of intravenous contrast material. Per convention, lesions with a mean attenuation of >130 Hounsfield units (HU) with an area of 1 mm^2 were included in the CAC score. The Agatston score (traditional calcium score) was calculated according to the method described by Agatston et al[11] using automated software (Circulation, Siemens Medical Solutions). In brief, the score is derived by measuring the plaque area and the maximum attenuation within each region of interest for each calcified coronary artery focus. The score is then calculated by multiplying the measured area of calcium per coronary segment by an attenuation coefficient based on the peak computed tomographic number (coefficient 1 for peak attenuation of 131 to 200 HU; coefficient 2 for peak attenuation of 201 to 300 HU; coefficient 3 for peak attenuation of 301 to 400 HU; and coefficient 4 for peak attenuation of ≥401 HU). The sum of the individual scores measured within the borders of each coronary artery was used to compute the final Agatston score. Patients were classified as having an Agatston score of 0 or >0 to indicate the presence of CAC.

Age, gender, race/ethnicity, and the Framingham risk score[12] were assessed as potential confounding variables between the telomere length and the presence of CAC. Race/ethnicity was classified as non-Hispanic white or non-white. The Framingham risk score was included as a measure of cardiovascular risk status. This score includes age, total cholesterol level, smoking status, high-density lipoprotein cholesterol level, systolic blood pressure, and treatment of hypertension. The participants were classified into 10-year risk categories of <10% and ≥10%.

Table 2
Association between telomere length and lifestyle characteristics and presence of coronary artery calcium (CAC)*

Variable	Telomere Length	OR	95% CI
Fruit and vegetable consumption			
High	Short	2.04	0.93–4.46
Low	Short	3.30	1.61–6.75
High	Long	1.00	—
Low	Long	1.20	0.61–2.37
Meat consumption			
High	Short	3.33	1.54–7.20
Low	Short	1.72	0.83–3.55
High	Long	0.87	0.44–1.74
Low	Long	1.00	—
Exercise			
High	Short	2.76	1.28–5.95
Low	Short	3.03	1.40–6.55
High	Long	1.00	—
Low	Long	1.34	0.68–2.65
Body mass index			
High	Short	4.30	1.95–9.50
Low	Short	2.40	1.12–5.14
High	Long	1.64	0.81–3.34
Low	Long	1.00	—
Social support			
High	Short	1.54	0.68–3.48
Low	Short	2.58	1.24–5.37
High	Long	1.00	—
Low	Long	0.73	0.37–1.44

* Adjusted for age, gender, race/ethnicity, and Framingham risk score.
CI = confidence interval; OR = odds ratio.

Bivariate comparisons were performed comparing the proportions using unadjusted chi-square analyses and mean values using Student's *t* test for gender-stratified demographic data. Unadjusted chi-square values were also used to compare the association between telomere length and CAC for the total sample and stratified by gender. Two types of adjusted logistic regression analyses were performed. The first tested the interaction of telomere length with the lifestyle characteristic variables in determining the association with the presence of CAC. In these regression analyses, the telomere length, fruit and vegetable consumption, meat consumption, and body mass index were used as continuous variables, and exercise and social support were split into 2 categories (greater than and less than the median) owing to their assessment using an ordinal scale. Second, adjusted logistic regression analyses, each using one of the 4-part telomere length and lifestyle characteristic variables, were used to determine the association between telomere length and lifestyle characteristics with the presence of CAC. These regression analyses were adjusted for age, race, gender, and Framingham risk score. Finally, separate logistic regression analyses were performed by gender, adjusting for age, race, and Framingham risk score. From

Table 3
Association between telomere length and lifestyle characteristics and presence of coronary artery calcium (CAC) in men*

Variable	Telomere Length	OR	95% CI
Fruit and vegetable consumption			
High	Short	2.78	0.85–9.06
Low	Short	3.82	1.40–10.44
High	Long	1.00	—
Low	Long	0.79	0.30–2.11
Meat consumption			
High	Short	4.73	1.56–14.31
Low	Short	2.28	0.81–6.43
High	Long	0.69	0.25–1.89
Low	Long	1.00	—
Exercise			
High	Short	3.71	1.33–10.35
Low	Short	7.98	2.34–27.25
High	Long	1.00	—
Low	Long	1.68	0.62–4.51
Body mass index			
High	Short	11.92	3.10–45.77
Low	Short	5.77	1.93–17.23
High	Long	3.44	1.16–10.26
Low	Long	1.00	—
Social support			
High	Short	1.80	0.48–6.78
Low	Short	3.43	1.23–9.59
High	Long	1.00	—
Low	Long	0.60	0.22–1.63

* Adjusted for age, race/ethnicity, and Framingham risk score.
CI = confidence interval; OR = odds ratio.

Table 4
Association between telomere length and lifestyle characteristics and presence of coronary artery calcium (CAC) in women*

Variable	Telomere Length	OR	95% CI
Fruit and vegetable consumption			
High	Short	1.44	0.46–4.47
Low	Short	2.97	1.00–8.79
High	Long	1.00	—
Low	Long	1.73	0.68–4.43
Meat consumption			
High	Short	2.20	0.70–6.94
Low	Short	1.31	0.45–3.80
High	Long	0.99	0.39–2.53
Low	Long	1.00	—
Exercise			
High	Short	2.07	0.61–7.05
Low	Short	1.57	0.53–4.64
High	Long	1.00	—
Low	Long	1.12	0.44–2.84
Body mass index			
High	Short	2.18	0.78–6.04
Low	Short	0.83	0.23–2.96
High	Long	0.82	0.31–2.17
Low	Long	1.00	—
Social support			
High	Short	1.37	0.47–4.00
Low	Short	1.80	0.58–5.63
High	Long	1.00	—
Low	Long	0.89	0.35–2.26

* Adjusted for age, race/ethnicity, and Framingham risk score.
CI = confidence interval; OR = odds ratio.

published data that suggested 15 subjects per predictor would be sufficient for these types of regression analyses, a minimum sample size of 120 subjects was necessary for these equations.[13]

Results

The demographic characteristics for the sample are listed in Table 1. Unadjusted chi-square values between telomere length and CAC demonstrated an association between shorter telomere length and CAC >0 for the total sample (47% vs 23%, $p < 0.0001$) and for men (61% vs 28%, $p < 0.01$) but not for women (31% vs 19%, $p = 0.08$).

Adjusted logistic regression testing for interactions between telomere length and lifestyle variables showed the interactions between telomere length and fruit and vegetable consumption ($p = 0.02$), meat consumption ($p = 0.04$), and social support ($p = 0.02$) were significantly associated with CAC. In contrast, the interactions between telomere length and exercise ($p = 0.67$) and body mass index ($p = 0.83$) were not significantly associated with the presence of CAC.

The results from the adjusted logistic regression analyses using the total sample are listed in Table 2. Regression analyses evaluating exercise and body mass index demonstrated an increased risk of elevated CAC for those with shorter telomere lengths, regardless of whether they exercised often or had a lower body mass index. In contrast, an increased risk of elevated CAC was seen only in those with the unhealthy lifestyle factor and short telomeres for fruit and vegetable consumption, meat consumption, and social support. Thus, eating more fruit and vegetables and less meat and having more social support seemed to attenuate the association between shorter telomeres and CAC. The results from the gender-stratified adjusted logistic regression analyses are presented in Tables 3 and 4. The results for the men followed a similar pattern to that seen for the total sample, but the odds ratios for women reach statistical significance only for low fruit and vegetable consumption and shorter telomere length (odds ratio 2.966, 95% confidence interval 1.002 to 8.786).

Discussion

The telomere length is a novel risk marker for coronary heart disease, with studies consistently associating shorter telomere lengths with increased risk.[1,8,14] Because the telomere length is known to reflect both accumulated environmental injury and genetic predisposition, it might be a better predictor than other more-conventional risk markers that only reflect a patient's current risk status. Although not currently used to asses risk in clinical practice, increasing evidence supporting its use and the improved ability to perform the test quickly and accurately using PCR methods from blood or buccal swabs might lead to its clinical use in the future. No previous study to our knowledge has evaluated this novel predictor with a marker of coronary atherosclerosis, taking into account the possible effect of lifestyle characteristics on this association. Our results suggest that

despite past accumulated injury leading to shorter telomere lengths, current healthy behaviors might help to decrease a person's risk of atherosclerosis. If true, this would provide further impetus to promote healthy behaviors as a method of decreasing a person's coronary heart disease risk, regardless of previous behavior or current risk status. Because not all healthy behaviors examined attenuated the relation, additional studies would also need to identify which behaviors could be the most effective in reducing risk.

The stratification by gender for the analyses showed that the results for men were consistent with the associations seen for the total sample; however, the results for women were not statistically significant, except for low fruit and vegetable consumption. This could have been because of the well-established gender differences in cardiovascular disease risk, with men having greater risk earlier in life.[15] This was reflected in our study, with women having less CAC and lower risk profiles, despite the similar mean ages of the samples. Additionally, human and animal studies have revealed greater telomerase activity and longer telomeres in women than in men and have suggested that estrogens might contribute to these gender differences.[16] Additional studies are necessary to evaluate the effect of gender on the association between telomere length and CAC, and the role of lifestyle factors in this relation.

Potential mechanistic explanations for the association between telomere length and atherosclerosis have been previously discussed.[17] One possibility is that the telomere length is not actively involved in the atherosclerotic process but is, instead, a biomarker that reflects the accruing burden of inflammation and oxidative stress that drives the process of atherosclerosis.[18–20] Conversely, it is known that excessive shortening of the telomere length leads to cell senescence; thus, a shorter leukocyte telomere length could be an index of decreased hematopoietic stem cell function. This could reflect a decreased ability of the bone marrow to supply functional endothelial progenitor cells to the circulation, with a subsequent decrease in the endothelial repair performed by these cells, leading to increased atherosclerosis.[21,22]

The present study had several limitations. First, because this was a cross-sectional sample, we could only evaluate the associations between these factors. The associations presented suggest the need for prospective studies to further evaluate these findings. Second, a number of other lifestyle characteristics could show relationships similar to the ones we have presented. The analyses were limited to the lifestyle factors evaluated in the sample that had a high enough proportion of subjects in the healthy and unhealthy groups to provide an adequate sample size for analysis. Third, our lifestyle variables were defined by self-report and thus could have been affected by recall bias. However, the recall bias was minimized by asking the participants about current behaviors instead of lifetime behaviors.

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