Inflammation and Not Cardiovascular Risk Factors Is Associated With Short Leukocyte Telomere Length in 13- to 16-Year-Old Adolescents


Objective—Short leukocyte telomere length (LTL) is associated with cardiovascular (CV) disease in adulthood. However, the biological basis of this association remains unclear. We sought to define early determinants of the association between CV disease and LTL in an adolescent population.

Methods and Results—One thousand eighty adolescents, aged 13 to 16 years and participating in the Ten Towns Heart Health Study, provided blood samples for DNA extraction and measurement of a range of CV risk factors. LTL was measured by real-time polymerase chain reaction. LTL was inversely associated with age ($P=0.04$), longer in females than in males ($P=0.03$), and longer in South Asians than in white Europeans ($P=0.01$). No associations were found between LTL and traditional CV risk factors. There was a significant and inverse association between LTL and inflammatory markers, including C-reactive protein ($P<0.001$) and fibrinogen ($P=0.001$). The associations between LTL and inflammatory markers were not affected by multiple adjustments for behavioral and metabolic factors.

Conclusion—High levels of inflammation are associated with shorter LTL from early adolescence; traditional CV risk factors have little association with LTL in adolescence. Inflammation in early life may play a causal role in the adult association between short LTL and CV disease. (Arterioscler Thromb Vasc Biol. 2012;32:2029-2034.)

Key Words: telomeres ▪ young ▪ inflammation ▪ C-reactive protein ▪ cardiovascular risk factors

Telomeres are long lengths of TTAGGG repeats, which cap the end of the chromosomes and protect them from degradation and fusion.1 By undergoing erosion with each replicative cycle, telomeres chronicle the replicative history of human somatic cells in vitro and in vivo.2 Cross-sectional studies in adults have described complex associations between leukocyte telomere lengths (LTLs) and clusters of traditional cardiovascular (CV) risk factors, including dyslipidemia, hypertension, diabetes mellitus, obesity, and smoking.3,4 Furthermore, shorter LTL has been associated with subclinical5 and clinical6 markers of atherosclerosis. The biological basis for these associations, however, remains unclear. Inflammation, a novel risk factor for CV disease, has been suggested as a common driver of both atherosclerosis and an increased rate of LTL shortening.7 A chronic inflammatory exposure may play a key role in coronary artery disease and other manifestations of atherosclerosis. Immune cells dominate early atherosclerotic plaques, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit acute coronary syndromes.8 Indeed, inflammatory markers such as C-reactive protein (CRP) and fibrinogen may improve the CV risk stratification compared with that provided by traditional CV risk factors (eg, cholesterol, smoking, diabetes mellitus).9 Simultaneously, a chronic increase in the systemic inflammatory burden could enhance the rate of telomere attrition in peripheral leukocytes by increasing the number of cell replications and by exposing the telomere sequences to higher levels of oxidative stress.7,10

In adult cross-sectional studies, it is difficult to explore the biological origins of the association between LTL and CV disease because of the long-term exposure of patients to a variable level of CV risk factors. In contrast, in the young, there is a considerably lower risk factor burden as well as a shorter exposure period, and this provides the opportunity to study early biological mechanisms that may account for the adult association between LTL and CV disease. We, therefore,

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2029
studied adolescents (13–16 years old) who took part in the third phase of the Ten Towns Heart Health Study to examine the association of LTL with traditional and novel markers of CV risk. The Ten Towns Heart Health Study was originally designed to examine levels of CV risk factor in adolescents from areas of England and Wales with high and low adult CV mortality and involved detailed characterization of a broad range of CV risk factors. We were, therefore, able to make appropriate adjustments for most of the environmental factors that have previously been associated with short LTL in adults.

Methods

Population

The Ten Town Heart Health Study included 1248 European and 90 South Asian adolescents (aged 13–16 years) recruited between 1998 and 2000 from 69 secondary schools in 10 towns across England and Wales.12 Ethical approval was received from the local research ethics committees, and written informed consent was obtained from all participants and their parents. Ethnicity was defined by appearance (European, South Asian, other) and cross-checked with parental place of birth. Height, weight, blood pressure, waist and hip circumferences, skinfold thicknesses, bioimpedance measurement (Bodystat 500: Bodystat Ltd, IoM), and puberty status (Tanner stage) were assessed using standard techniques by a trained field team (4 observers) who visited each town in turn.13 Participants provided questionnaire-based information on how their physical activity levels compared with those of their peers, with 5 grades (much less active, slightly less active, similar, more active, much more active).12,13 Parental information on occupation was coded using the Registrar General’s 1990 Occupational Classification. Cigarette smoking status was established by a questionnaire regarding current and lifelong smoking to separate participants into current smokers (≥1 cigarette per day currently), past smokers (history of previous but not current cigarette smoking), and nonsmokers. Salivary cotinine measurement was carried out; levels ≥14.1 ng/mL were used to reclassify past or nonsmokers as current smokers.14 A blood sample was collected after an overnight fast for DNA extraction and measurements of traditional and novel markers of CV risk.

Blood Samples

Blood samples were frozen (−20°C) within 6 hours of collection and transferred to a central laboratory for analysis within 2 weeks or for long-term storage at −70°C. Biochemical assays were performed using standardized methods as reported in online-only Data Supplement.

LTL Assay

DNA was extracted from peripheral blood cells by a salting-out procedure as previously described.14 Telomere lengths were measured using a validated quantitative polymerase chain reaction–based method.14 Details of the techniques, together with their reproducibility, have been reported in the online-only Data Supplement. Final LTL measure was expressed as the ratio between the number of telomere repeats and single-copy gene copies (T/S ratio) presented in each sample, as specified in the online-only Data Supplement. All analyses were processed in blinded fashion.

Statistical Analysis

Statistical analyses were carried out using STATA/SE software (Stata/SE 10 for Windows; StataCorp LP, College Station, TX). Variables were checked for normality and log transformed where necessary. Means and SD (or geometric means and SDs for log-transformed variables) for adiposity and blood analyses were presented unadjusted. Means and differences in LTL were adjusted for age, sex, ethnicity, and town using the regress and lincom procedures within STATA. Estimates of mean LTL by quintiles of CRP and fibrinogen adjusted for age, sex, ethnicity, and town were obtained using the same procedures. Associations among anthropometry, blood pressure, blood analytes, and telomere length were examined using linear regression models adjusted for age, sex, ethnicity, and town.

Results

Anthropometric, blood, and LTL measurements were available from 1080 subjects (992 white Europeans, 73 South Asians, and 15 from other ethnicities). Their clinical characteristics are shown in Table 1 with associations between each variable and LTL after adjustment for age, ethnicity, sex, and town. Measures of LTL were normally distributed (Figure I in the online-only Data Supplement). Mean LTL was 1.30 (range 0.65–2.32). A negative linear association was found between LTL and age (β=-0.016, 95% CI [-0.032 to -0.001], P=0.04), whereas there were no associations between LTL and circulating levels of traditional CV risk factors including dyslipidemia, hypertension, insulin resistance, and obesity (Table 1). However, there was a strong inverse association between LTL and circulating levels of inflammatory markers, CRP (β=-0.026, 95% CI [-0.041 to -0.011], P<0.001) and fibrinogen (β=-0.025, 95% CI [-0.040 to -0.010], P=0.001) (Table 1). No sex interaction was found for these associations (Table 1 in the online-only Data Supplement). There was a graded decline in mean LTL with increasing levels of CRP, and mean LTL was significantly shorter in subjects in the top quintile of CRP levels compared with the first quintile (mean difference T/S ratio 0.09, P<0.001) (Figure). Similarly, for fibrinogen (Figure) the mean LTL was significantly shorter in subjects in the top quintile compared with the first quintile (mean difference T/S ratio 0.07, P=0.003). CRP and fibrinogen levels were strongly associated with each other (r=0.6; P<0.001). The associations among CRP, fibrinogen, and LTL were not mutually adjusted because these 2 strongly intercorrelated variables are likely to represent the same biological pathway. No differences were found between groups of towns with high and low adult CV mortality. Males had shorter LTL than females (mean difference T/S ratio 0.03, P=0.03) (Table 2), whereas subjects of South Asian ethnicity showed longer LTL than white Europeans (mean difference T/S ratio 0.10, P=0.01) (Table 2). Current and previous smokers tended to have shorter LTL than nonsmokers (P=0.06 for a difference between the 3 smoking groups; Table 2) with and without correction for cotinine levels.

The strength of the inverse association between LTL and inflammatory markers was not affected by adjustments for behavioral factors (physical activity and smoking status), metabolic factors (body mass index, parameters of metabolic syndrome), circulating levels of antioxidant molecules (ie, folate, vitamin C), and socioeconomic and puberty status (Tanner stage) (Table III in the online-only Data Supplement). The sex and ethnic differences in LTL were not affected by adjustment for anthropometric parameters, Tanner stage, smoking status, levels of physical activity, socioeconomic status, and CV risk factors (data not shown).

Analyses were repeated in the white European participants alone (Table I in the online-only Data Supplement), as well as
Vitamin C (49.9 23.6 mol/L)*  
Homocysteine (8.0 1.3 mol/L)*  
Fibrinogen (g/L)*  
Insulin (mU/L)*  
Triglycerides (mmol/L)*  
HDL cholesterol (mmol/L)  
LDL cholesterol (mmol/L)  
Pulse pressure (mm Hg)  

19 Although other cross-sectional studies described an inverse association between LTL and inflammatory markers in middle-age populations, our findings suggest that this association begins much earlier in life and persists for levels of CRP and fibrinogen below those associated with CV risk elevation in adults. This suggests that even physiological variations in inflammatory states could have an effect on the CV biology (ie, LTL) in the young. A recent meta-analysis supports our findings, demonstrating a continuous relationship between CRP and CV outcome, even after adjustment for other traditional CV risk factors.21

Two recent studies have measured LTL in children and adolescents. In a study specifically based on obese children (2–17 years old) compared with their lean peers, Buxton et al22 reported significantly shorter LTL in obese children. Their findings contrast both with our study, which observed no relationship between adiposity and LTL across the population, and with the study by Zhu et al,23 which also reported null associations between biochemical and anthropometric measures of adiposity and LTL in a cohort of 667 young adolescents. Although the studies by Buxton et al22 and Zhu et al23 measured LTL in children and young adolescents, neither examined the impact of inflammation or other CV risk factors on LTL.

Atherosclerosis is an age-related systemic disease that begins from childhood24 and is largely driven by chronic inflammation and Telomere Length in Adolescence
inflammatory and oxidative stress exposure. It has been suggested that the shortened LTL often observed in adult patients who suffer from atherosclerosis may be the result of a continuous stimulus to the recruitment, differentiation, and replication of new inflammatory cells as well as a higher level of oxidative stress-mediated damage to the telomere sequence. Our findings support this hypothesis and suggest that the association observed in adults between short LTL and CV disease is a late reflection of biological pathways which could act from early adolescence. Furthermore, in our study the association between inflammation and LTL is not explained by environmental or behavioral factors (e.g., socioeconomic class, levels of physical activity, smoking status), previously associated with shortened LTL. This suggests that heritable factors, influencing the level of inflammatory response to environmental exposure, could represent early determinants of the association among short LTL, inflammation, and future risk of CV disease.

LTL was shorter in males than females. This finding is in agreement with that of Zhu et al, who recently reported longer LTL in adolescent females compared with males using a smaller cohort of healthy white and black adolescents. Interestingly, our data suggest that this sex difference is not influenced by exposure to different levels of inflammation and other CV risk factors. This is potentially important as it has been hypothesized that the longer LTL recorded in females is mediated by the anti-inflammatory and antioxidative effects of endogenous estrogens, effects that may also account for the lower incidence of cardiovascular diseases recorded in premenopausal women. In contrast to this hypothesis, we did not find a sex interaction for the association between LTL and inflammatory markers, and adjustment for Tanner stage did not materially affect this association as well as the sex difference in LTL. Such findings, together with the longitudinal findings from the Bogalusa Heart Study that did not find differences in the rate of telomere shortening between females and males, suggest that estrogen exposure hardly accounts for the sex gap in LTL and that other factors may be involved. As robust correlations in LTL have been described between fathers and daughters, between mothers and sons and daughters, and among siblings, it has been suggested that gene variance on the X chromosome influences telomere length dynamic. Our results would be consistent with this hypothesis and further suggest that factors accounting for this X-linked heritance are likely to exert their effects during the prepubertal period, independently from the CV risk factor burden.

We describe, for the first time, longer LTL in young South Asian adolescents compared with their white European counterparts. The biological impact of this, however, remains unexplored, and interpretation should be cautious as there were a limited number of South Asian adolescents in our cohort and there is no evidence as of yet that LTL predicts ethnic difference in CV outcomes.

The Ten Towns Health Heart Study has a number of strengths for the investigation of the LTL biology and its relationship with CV risk factors and inflammation. First, the cohort is relatively large and representative of the UK population, excluding the influence of factors affecting telomere length across populations. Data on a wide range of established and novel CV disease risk factors and potential confounders were measured, allowing the independent role of many parameters to be assessed. In addition, the impact of a long-term exposure to a range of CV risk factors, which is hard to quantify in adult
studies, is minimized. This has potential to identify the initial and prolonged biological pathways, accounting for the adult association between short LTL and CV disease.

Our report also has important limitations. Causality cannot be inferred from the observed cross-sectional association between inflammatory markers and LTL. However, although in adults reverse causality is a possibility as short telomeres may activate production of proinflammatory cytokines, this is an unlikely explanation of our findings as LTLs in adolescents are longer than those associated with this telomere-induced inflammatory activation in adults. The quantitative polymerase chain reaction-based assay (as well as other techniques commonly used to measure LTL in large, population-based studies) provides only the average telomere length across all leukocytes. Therefore, it is not possible to compare telomere length between different leukocyte subpopulations. Nevertheless, several reports have now demonstrated that telomere length is highly synchronized between different cells and tissues in fetuses, newborns, and, more in general, at any age. This is because of the high interindividual variability in telomere length at birth and thereafter, which far exceeds the variations among cell types within the individual. Indeed, in healthy subjects, as well as in disease conditions, the presence of a relatively long (or short) telomere length in one cell type forecasts the presence of long (or short) telomere length in other cellular phenotypes. The small range and young age of our population preclude evaluation of long-term effects, for example, the impact of smoking and prolonged biological pathways, accounting for the adult cumulative burden of inflammation even before puberty. Although clinical problems from CV disease do not usually appear before middle age, the association between short LTL and inflammatory markers in early adolescence may represent a possible biological pathway, accounting for the relationship between short LTL and atherosclerosis seen in adult studies. Traditional CV risk factor levels do not appear to be major contributors to this pathophysiological process in youth. Treatment approaches, aiming to reduce the systemic burden of inflammation, may modify or even prevent the evolution of this vascular aging process. As LTL records long-term inflammatory exposure, it could be a useful biomarker to reveal the cumulative burden of inflammation even before puberty.

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Disclosures

None.

References


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Inflammation and not cardiovascular risk factors is associated with short leukocyte telomere length in 13 to 16 years old adolescents

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**Biochemical assays**

Total serum cholesterol and high-density lipoproteins were measured using a Hitachi 747 automated analyzer (Roche Diagnostics Corp, Indianapolis, IN)\(^1\). LDL was calculated using the Friedewald equation\(^2\). Plasma glucose was measured in a fluoride oxalate sample with a Falcor 600 automated analyser and serum insulin using a specific ELISA assay which does not cross-react with proinsulin\(^3\). C reactive protein was determined with a high sensitive enzyme immunoassay with DAKO reagents\(^4\). Plasma levels of tissue Plasminogen Activator (tPA) and von Willebrand factor (vWF) were measured with ELISAs (Biopool AB and DAKO, respectively)\(^5\). Clottable fibrinogen was measured with the Clauss method in blood anticoagulated with 0.109 mol/L trisodium citrate (9:1 vol:vol)\(^6\). Serum total homocysteine was measured by a modified automated assay, based on pre-column derivatisation with monobromobimane, followed by reverse phase high performance liquid chromatography with fluorescence detection\(^7\). Serum folate levels were determined by a microbiological assay with the use of a chloramphenicol resistant strain of Lactobacillus casei\(^8\). Plasma vitamin C and was measured with standard high performance liquid chromatography (HPLC) methods; samples for vitamin C analysis were pretreated with metaphosphoric acid at the point of collection and then snap-frozen with dry ice\(^9\). The assessment of insulin resistance was based on the homeostasis model assessment (HOMA) equation (glucose × insulin/22.5)\(^10\).

**Leukocyte telomere length assay**

Telomere lengths were measured using a validated quantitative polymerase chain reaction (Q-PCR)-based method\(^11\). In each sample, LTL was measured with a quantitative PCR-based technique that compares telomere repeat sequence copy number to single-copy gene (36b4) copy number. In each reaction the number of telomere repeats and single copy gene copies (SCG) were determined in comparison with a reference sample and the final LTL measure resulted from the ratio between telomere repeats to SCG copies (T/S ratio). The same reference DNA was used in all runs to allow comparison of the results in different runs. The reference DNA was obtained by random selection of one clinical sample from a study previously conducted in our lab. It was the same sample used in
previous publications\textsuperscript{11-14}. The same method was used in order to extract and standardize the DNA concentration of the reference and study samples. Every sample was run in duplicate and the mean data were used for the calculations. The primers used for the telomere and the SCG amplification were as in Cawthon's report\textsuperscript{15}. In the telomere PCR, primer concentrations were 135/900 nM (forward/reverse) and the cycling profile: 95°C incubation for 10 min, followed by 22 cycles of 95°C for 15 s and 58°C for 120 s. In the SCG PCR, primer concentrations were 300/500 nM and the cycling profile: 95°C incubation for 10 min, followed by 30 cycles of 95°C for 15 s and 58°C for 60 s. For both the telomere and the SCG PCR the final reaction volume was 25 µl consisting of 1× SYBR Green, 1× qPCR mix (2× SensiMix NoRef DNA kit, Quantace, London, UK), 30 ng of template, and the respective primer concentrations. All PCRs were performed on a Rotor-Gene 6000 machine (Corbett Research Ltd, Cambridge, UK), and the raw data were analysed using comparative quantification analysis (Rotor-Gene 6000 software, Corbett Research Ltd, Cambridge, UK), as previously described. The specificity of all amplifications was assessed by melting curve analysis. To test the reproducibility of our qPCR technique we adopted a commonly used approach which is based on running a subset of DNA samples in duplicate on two different days\textsuperscript{12, 13, 15-26}. Using 20 randomly selected DNA samples from our cohort, the inter-assay coefficient of variation was 3.27%; the correlation coefficient between the average T/S ratio determined by the first and second runs was 0.93. The estimate of the coefficient of variation, though potentially an underestimate of the true coefficient of variation based on separate DNA extractions, is lower than that of several previous studies using similar methods\textsuperscript{13, 15-26}.
References


Author Contribution

Design of original survey and participant recruitment: PHW, DGC; Study design: SM, SEH, JED, PHW, DGC, TVZ, ST, NK, FDA; Telomere assay design and set up: SHE, KS; Telomere assays: SM; Biochemical Assays: GDOL, AR; Statistical analysis: CMN; Data interpretation: SM, JED, PHW, DGC, SEH, TVZ, GDOL, ST, NK, FDA; Manuscript preparation: SM, PHW, JED; Manuscript critical revision: SM, SEH, JED, PHW, DGC, TVZ, ST, NK, FDA, KS, GDOL, AR.
### Supplementary Table I. Associations between Telomere length (T/S ratio) and other variables by gender

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference in T/S ratio for one SD / log SD† increase in variable (95% CI)</td>
<td>p(linear assocs)</td>
<td>Difference in T/S ratio for one SD / log SD† increase in variable (95%)</td>
<td>p(linear assocs)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.005 (-0.018, 0.027)</td>
<td>0.69</td>
<td>0.017 (-0.006, 0.041)</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>-0.014 (-0.034, 0.006)</td>
<td>0.17</td>
<td>0.002 (-0.021, 0.024)</td>
<td>0.88</td>
</tr>
<tr>
<td>Waist circumference (cm)*</td>
<td>-0.008 (-0.028, 0.013)</td>
<td>0.46</td>
<td>0.003 (-0.019, 0.026)</td>
<td>0.77</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)*</td>
<td>-0.013 (-0.033, 0.007)</td>
<td>0.21</td>
<td>-0.001 (-0.024, 0.022)</td>
<td>0.93</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>-0.012 (-0.032, 0.008)</td>
<td>0.24</td>
<td>-0.006 (-0.028, 0.017)</td>
<td>0.63</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.005 (-0.016, 0.026)</td>
<td>0.64</td>
<td>0.015 (-0.008, 0.037)</td>
<td>0.20</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.015 (-0.005, 0.036)</td>
<td>0.13</td>
<td>0.008 (-0.014, 0.030)</td>
<td>0.48</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>-0.004 (-0.025, 0.016)</td>
<td>0.67</td>
<td>0.012 (-0.010, 0.034)</td>
<td>0.29</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.009 (-0.011, 0.030)</td>
<td>0.39</td>
<td>0.007 (-0.016, 0.030)</td>
<td>0.54</td>
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<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.009 (-0.011, 0.029)</td>
<td>0.38</td>
<td>0.004 (-0.019, 0.026)</td>
<td>0.75</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)*</td>
<td>-0.004 (-0.025, 0.017)</td>
<td>0.71</td>
<td>-0.013 (-0.036, 0.011)</td>
<td>0.29</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.015 (-0.037, 0.007)</td>
<td>0.19</td>
<td>0.019 (-0.006, 0.043)</td>
<td>0.13</td>
</tr>
<tr>
<td>Insulin (mU/L)*</td>
<td>-0.010 (-0.030, 0.010)</td>
<td>0.34</td>
<td>-0.013 (-0.035, 0.009)</td>
<td>0.25</td>
</tr>
<tr>
<td>Insulin resistance (HOMA)*</td>
<td>-0.013 (-0.035, 0.008)</td>
<td>0.23</td>
<td>0.018 (-0.006, 0.041)</td>
<td>0.14</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)*</td>
<td>-0.020 (-0.040, 0.000)</td>
<td>0.05</td>
<td>-0.032 (-0.055, -0.010)</td>
<td>0.01</td>
</tr>
<tr>
<td>Von Willebrand factor (IU/dL)*</td>
<td>-0.016 (-0.036, 0.004)</td>
<td>0.11</td>
<td>0.001 (-0.021, 0.023)</td>
<td>0.90</td>
</tr>
<tr>
<td>Tissue plasminogen activator (ng/mL)*</td>
<td>0.014 (-0.007, 0.034)</td>
<td>0.19</td>
<td>-0.005 (-0.028, 0.017)</td>
<td>0.64</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)*</td>
<td>-0.002 (-0.023, 0.018)</td>
<td>0.81</td>
<td>0.009 (-0.014, 0.032)</td>
<td>0.43</td>
</tr>
<tr>
<td>Fibrinogen (g/L)*</td>
<td>-0.014 (-0.034, 0.006)</td>
<td>0.17</td>
<td>-0.034 (-0.057, -0.012)</td>
<td>0.00</td>
</tr>
<tr>
<td>Vitamin C (µmol/L)</td>
<td>0.002 (-0.020, 0.025)</td>
<td>0.84</td>
<td>-0.009 (-0.035, 0.016)</td>
<td>0.46</td>
</tr>
<tr>
<td>Folate (nmol/L)*</td>
<td>0.006 (-0.014, 0.026)</td>
<td>0.56</td>
<td>0.012 (-0.011, 0.034)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

* Geometric mean and geometric SD and difference per log SD change presented for log transformed variables

Adjusted for age, ethnicity and town

Missing values: Sum of skinfolds (n=27)
**Supplementary Table II.** Associations between Telomere length (T/S ratio) and other variables – for white Europeans only

<table>
<thead>
<tr>
<th>White Europeans Variable (N=992)</th>
<th>Mean/SD</th>
<th>Geometric mean/SD</th>
<th>Difference in T/S ratio for one SD/Log SD† increase in variable (95% CI)</th>
<th>p(linear assocs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.1/0.6</td>
<td>0.016 (-0.033, 0.000)</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.5/8.5</td>
<td>0.015 (-0.004, 0.033)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>20.7/1.2</td>
<td>-0.006 (-0.022, 0.010)</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)*</td>
<td>69.8/1.1</td>
<td>-0.004 (-0.020, 0.012)</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Sum of skinfolds (mm)*</td>
<td>46.2/1.5</td>
<td>-0.008 (-0.025, 0.010)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Fat mass %</td>
<td>25.9/6.5</td>
<td>-0.013 (-0.031, 0.005)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>121.0/13.4</td>
<td>0.010 (-0.006, 0.026)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>67.0/7.2</td>
<td>0.012 (-0.004, 0.027)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>54.0/11.3</td>
<td>0.004 (-0.012, 0.021)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.2/0.7</td>
<td>0.009 (-0.007, 0.025)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.3/0.6</td>
<td>0.007 (-0.009, 0.022)</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.5/0.3</td>
<td>0.014 (-0.002, 0.030)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/L)*</td>
<td>0.9/1.4</td>
<td>-0.008 (-0.025, 0.008)</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0/0.5</td>
<td>0.001 (-0.016, 0.019)</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Insulin (mU/L)*</td>
<td>8.9/1.6</td>
<td>-0.010 (-0.025, 0.006)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Insulin resistance (HOMA)*</td>
<td>0.7/1.1</td>
<td>0.002 (-0.015, 0.018)</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (ng/L)*</td>
<td>0.2/3.3</td>
<td>-0.022 (-0.038, -0.007)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Von Willebrand factor (IU/dL)*</td>
<td>99.9/1.4</td>
<td>-0.011 (-0.027, 0.006)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Tissue plasminogen activator (ng/mL)*</td>
<td>5.3/1.5</td>
<td>0.006 (-0.010, 0.021)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (µmol/L)*</td>
<td>8.0/1.3</td>
<td>0.001 (-0.014, 0.017)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)*</td>
<td>2.5/1.2</td>
<td>-0.023 (-0.039, -0.007)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (µmol/L)*</td>
<td>51.6/23.1</td>
<td>-0.001 (-0.019, 0.016)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Folate (nmol/L)*</td>
<td>14.3/1.7</td>
<td>0.010 (-0.006, 0.026)</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

* Geometric mean and geometric SD and difference per log SD change presented for log transformed variables

Adjusted for sex, age and town

Missing values: Sum of skinfolds (n=25)
**Supplementary Table III.** Associations between Telomere length (T/S ratio) CRP and Fibrinogen adjusted for other factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Additionally adjusted for</th>
<th>Difference in T/S ratio for log SD increase variable (95% CI)</th>
<th>p (linear assoc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>No additional adjustments</td>
<td>-0.026 (-0.041, -0.011)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Physical activity (5 level variable)</td>
<td>-0.028 (-0.043, -0.012)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Smoking status corrected for cotinine</td>
<td>-0.026 (-0.041, -0.011)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Folate, Vitamin C</td>
<td>-0.026 (-0.041, -0.011)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.026 (-0.042, -0.010)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>WC§, triglycerides, HDL, SBP¶, Glucose</td>
<td>-0.026 (-0.042, -0.010)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>All of the above</td>
<td>-0.026 (-0.042, -0.010)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>No additional adjustments</td>
<td>-0.025 (-0.040, -0.010)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Physical activity (5 level variable)</td>
<td>-0.025 (-0.040, -0.010)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Smoking status corrected for cotinine</td>
<td>-0.025 (-0.040, -0.010)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Folate, Vitamin C</td>
<td>-0.026 (-0.041, -0.011)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.025 (-0.041, -0.009)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>WC§, triglycerides, HDL, SBP¶, Glucose</td>
<td>-0.024 (-0.040, -0.009)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>All of the above</td>
<td>-0.023 (-0.039, -0.007)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

All differences adjusted for sex, age, ethnicity and town

§ WC: Waist Circumference

¶ SBP: Systolic Blood Pressure
Supplementary Figure I. Distribution of telomere length (T/S ratio) - LTL were normally distributed.