Association Between Shortened Leukocyte Telomere Length and Cardiometabolic Outcomes
Systematic Review and Meta-Analysis

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Background—Telomeres are repetitive, gene-poor regions that cap the ends of chromosomes.1 Folding back on themselves to form a protective loop, they help stabilize chromosomes through preventing degradation, end-to-end fusion, and abnormal recombination of DNA strands.2 With each cell cycle telomeres shorten 30 to 200 nucleotides.3 This process is further accelerated as a result of oxidative stress and chronic inflammation.4,5 After telomeres decrease in size to a critical length, they are no longer able to serve their protective purposes. Consequently, cell cycle arrest (senescence) or apoptosis is activated.6 Because increased cellular senescence and oxidative stress are both key indicators of aging, it has been suggested recently that a shortened average telomere length could serve as a biomarker for aging and age-related diseases.7

Cardiovascular disease (CVD) and type 2 diabetes mellitus (T2D) are 2 disorders clearly related to age and a reduced life span.8 The incidence of these cardiometabolic outcomes demonstrates great interindividual variability within the same age group, suggesting that chronological age is not a precise measure of health status.9 There is therefore great use in identifying a biomarker that could provide further information about one’s cardiometabolic health in addition to (or in place of) chronological age, as it would aid in both the prediction and the prevention of disease. Telomere length may be one such biomarker.

To date, studies of the association between leukocyte telomere length (LTL), which reflects telomere length throughout the body,10 and cardiometabolic outcomes have yielded conflicting results. For example, some studies have reported a significant association between telomere length and stroke,11-14 whereas others have failed to demonstrate any such association.15-19 This inconsistency, which is also seen in studies investigating

Key Words: aging ■ diabetes mellitus, type 2 ■ myocardial infarction ■ stroke
other cardiometabolic outcomes, indicates that individual studies may not be statistically powered to detect true associations because of inadequate sample sizes. Furthermore, unstandardized laboratory techniques, different study designs, and ethnic diversity within study patient populations have also been suggested as plausible explanations for heterogeneous results.20,21

The primary objective of this systematic review and meta-analysis is to provide insight into the use of LTL as a biomarker of aging through a comprehensive assessment of the relationship between shortened LTL and the cardiometabolic outcomes of stroke, myocardial infarction (MI), and T2D. Secondary outcomes investigated include coronary artery disease (CAD), CVD-related death, and a major adverse cardiac event (MACE) composite outcome.

Methods

Eligibility Criteria

Articles deemed eligible for inclusion into the systematic review reported a hazard ratio or odds ratio (OR) for the association between LTL and ≥1 of the following outcomes: stroke, MI, T2D, CAD, CVD-related death, or MACE composite. CAD was defined as angina or a nonfatal ischemic heart disease composite (International Classification of Disease-Tenth Revision; codes I20–I25 or equivalent). MACE was defined as stroke, MI or CVD-related death. Both cross-sectional and prospective studies were selected for inclusion. If multiple publications reported the same outcome in identical populations, only the most recent publication was included. Publications were excluded if telomere length was not measured in leukocytes. No restrictions were placed on sample size, language of publication, date of publication, or publication status.

Information Sources and Search Strategy

Articles were accessed through OVID from the MEDLINE (1966 to present) and EMBASE (1980 to present) electronic databases. Limitations on the search restricted citations to only those including humans. Key MeSH terms used in the search strategy included: telomere, MI, stroke, diabetes mellitus, and death. See Tables I and II in the Data Supplement for complete search strategy for both databases. The last search was run on September 9, 2013. To identify further citations, the reference lists of articles retrieved were also hand searched.

Study Selection, Data Collection, and Data Items

Two reviewers, M.D. and S.R., independently selected studies for full-text review through title and abstract screening of citations retrieved from all sources. Full-text screening for final inclusion into the systematic review was also performed independently by both reviewers. Cohen’s unweighted $\kappa$ was used to evaluate agreement between both reviewers at each screening stage, and disagreements were resolved through consensus.

A data abstraction sheet was designed and piloted with 10 randomly selected studies. M.D. and S.R. independently extracted data pertaining to (1) study type, (2) patient baseline characteristics, (3)
LTL measurement technique, (4) study quality indicators, and (5) ORs or hazard ratios and the associated 95% confidence interval (CI). Disagreements were resolved through consensus.

**Risk of Bias in Individual Studies**

Risk of bias was independently assessed at the outcome level using an adapted version of the Newcastle–Ottawa Scale. Briefly, case–control and cohort studies were scored in 3 separate categories: selection, comparability, and exposure/outcome. Overall, each study received a rating from 0 to 8 stars depending on the likelihood of bias. A priori we established that 0 to 2, 3 to 5, and 6 to 8 stars would be considered at high, moderate, and low risk of bias, respectively.

**Summary Measures, Synthesis of Results, and Risk of Bias Across Studies**

The main summary measure was the pooled OR and 95% CI of a cardiometabolic outcome per-SD decrease in LTL. Cardiometabolic outcomes are relatively rare events and as such we treated hazard ratios as approximates of ORs. As described in the Methods in the Data Supplement, an effort was made to convert per-SD decrease when associations were reported based on quintile comparisons of LTL (ie, shortest versus longest quintile). Only the most adjusted effect measures were used so as to account for confounding.

The pooled OR was computed using the generic inverse variance method. This method weighs each study according to the inverse of the variance of the effect estimate to minimize uncertainty in the pooled effect estimate. Heterogeneity was assessed using the Cochran Q test and considered to be significant if $P<0.05$. In addition, $I^2$ was used as a measure of the portion of total variation in estimates that was because of heterogeneity. High heterogeneity was defined as $I^2>50\%$, whereas moderate and low heterogeneity were defined as $<50\%$ and $25\%$, respectively. Pooled summary estimates were initially calculated using the fixed effect model; however, if significant heterogeneity was observed, a random effects model was alternatively used. To assess for publication bias across studies inverted funnel plots were created for each outcome and visually inspected for asymmetry. A priori subgroup analyses based on study type, LTL measurement technique, sample size, and population ethnicity were conducted to examine possible sources of heterogeneity. Sensitivity analyses, also specified a priori, were conducted to observe the impact of removing studies at high or moderate risk of bias, and studies using highly variable LTL measurement techniques (interassay coefficient of variation $>10\%$). Statistical analyses were conducted using Review Manager (v5.2). All reported $P$ values were 2-sided.

**Results**

**Selection and Characteristics of Included Studies**

As shown in Figure 1, the electronic database search of MEDLINE and EMBASE resulted in the identification of 3382 relevant citations. A total of 2112 records remained after duplicate citations were removed and 2023 of these were excluded after title and abstract review for not meeting inclusion criteria. The full-text review of the 89 remaining articles yielded 27 publications for inclusion into the systematic review. No additional citations were retrieved from searching reference lists. Key reasons for exclusion included no cardiometabolic outcomes of interest measured (58), telomere length not obtained from leukocytes (2), and multiple publications of the same data set (2). A Cohen’s unweighted $k$ of 0.83 was achieved signifying good agreement between both reviewers. Among the 27 included publications in the systematic review, 1 was excluded from quantitative meta-analysis because of not providing enough information to calculate an appropriate OR and 95% CI. When necessary, authors of included studies were contacted for further information with respect to study characteristics or reported results.

Tables 1 to 3 describe the characteristics of included studies assessing stroke, MI, and T2D. Studies assessing CAD, CVD-related death, and MACE are presented in Tables III to V in the Data Supplement. One publication consisted of both a case–control and cohort study and therefore a total of 12 case–control and 15 cohort studies were included into the meta-analysis. Participants from the Cardiovascular Health Study and the Physicians Health Study were both included in multiple publications; however, different outcomes were reported. Several studies investigated LTL in specific ethnic groups. European white was the ethnic group studied in the majority of the studies.

**Table 1. Characteristics of Included Studies Assessing the Association Between LTL and Stroke**

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up, y</th>
<th>Events/Nonevents, n</th>
<th>Hospital vs Population Based</th>
<th>Average Age, y</th>
<th>Comorbidity</th>
<th>Ethnicity</th>
<th>LTL Assay</th>
<th>CV,* %</th>
<th>S</th>
<th>C</th>
<th>E/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ding et al11</td>
<td>Case–control</td>
<td>...</td>
<td>1309/1309</td>
<td>Population</td>
<td>66</td>
<td>...</td>
<td>Asian</td>
<td>qPCR</td>
<td>1.3</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Jiang et al15</td>
<td>Case–control</td>
<td>...</td>
<td>150/150‡</td>
<td>Population</td>
<td>51</td>
<td>...</td>
<td>Asian</td>
<td>qPCR</td>
<td>6.7</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Schürks et al17</td>
<td>Case–control</td>
<td>...</td>
<td>504/504§</td>
<td>Population</td>
<td>61</td>
<td>...</td>
<td>Mixed</td>
<td>qPCR</td>
<td>22</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Zee et al18</td>
<td>Case–control</td>
<td>...</td>
<td>259/259II</td>
<td>Population</td>
<td>62</td>
<td>...</td>
<td>Mixed</td>
<td>qPCR</td>
<td>&lt;2.0</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Zhang et al19</td>
<td>Case–control</td>
<td>...</td>
<td>503/1801</td>
<td>Hospital</td>
<td>60</td>
<td>...</td>
<td>Asian</td>
<td>qPCR</td>
<td>6.4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Ding et al11</td>
<td>Cohort</td>
<td>5.0</td>
<td>137/721</td>
<td>Hospital</td>
<td>60</td>
<td>Previous stroke</td>
<td>Asian</td>
<td>qPCR</td>
<td>1.3</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fitzpatrick et al12</td>
<td>Cohort</td>
<td>7.0</td>
<td>42/357</td>
<td>Population</td>
<td>74</td>
<td>...</td>
<td>Mixed</td>
<td>SB</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fyhquist et al13</td>
<td>Cohort</td>
<td>&gt;4.0</td>
<td>43/1228</td>
<td>Hospital</td>
<td>64</td>
<td>LVH</td>
<td>Mixed</td>
<td>SB</td>
<td>3.7</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Willeit et al14</td>
<td>Cohort</td>
<td>4.4</td>
<td>46/754</td>
<td>Population</td>
<td>63</td>
<td>...</td>
<td>White</td>
<td>qPCR</td>
<td>2.4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Yang et al17</td>
<td>Cohort</td>
<td>5.0</td>
<td>NR</td>
<td>Population</td>
<td>53</td>
<td>Hypertensive</td>
<td>Asian</td>
<td>qPCR</td>
<td>6.8</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation; LTL, leukocyte telomere length; LVH, left ventricular hypertrophy; NOS, Newcastle–Ottawa Scale; NR, not reported; qPCR, quantitative polymerase chain reaction; and SB, southern blot.

*CV=SD/mean of replicates run at different time points.
†Newcastle–Ottawa Scale: C, comparability (scored out of 2); E/O, exposure/outcome (scored out of 3); and S, selection (scored out of 4).
‡Controls are matched siblings.
§Women only; ‖men only.
predominantly reported, followed by Asian. Only 1 study presented effect measures stratified based on different ethnicities.\textsuperscript{30} All studies enrolled a similar amount of men and women, except for 5 that were sex specific.\textsuperscript{16,18,25,30,35} Quantitative polymerase chain reaction (qPCR) was the primary method of telomere measurement, with 4 studies using the Southern blot technique.\textsuperscript{12,13,24,34} Reported mean CVs ranged from 1.3% to 22%. Each study adjusted their reported effect measure for a variety of confounding variables and these are described in Tables VI to XI in the Data Supplement.

**Risk of Bias Within Studies**
The risk of bias assessment is presented at the outcome level in Tables 1 to 3. The majority of included studies had a low risk of bias according to the Newcastle–Ottawa Scale quality score. Two studies did not adjust for age in the experimental design and analysis,\textsuperscript{36,37} and thus were considered at risk of bias given the strong relationship between LTL and age. Lack of blinding of laboratory technicians and the use of highly specific patient populations were considered as further sources of bias.

**Primary Outcomes: Stroke, MI, and T2D**
A consistent positive association between per-SD decrease in LTL and all 3 primary cardiometabolic outcomes was observed (Figure 2). The 10 studies reporting stroke had a pooled OR of 1.21 (95% CI, 1.06–1.37) and displayed significant heterogeneity ($I^2=68\%$; $P<0.01$) when meta-analyzed using a random effects model. A more modest summary OR was identified when combining the 6 studies that reported on MI using a random effects model (OR, 1.24; 95% CI, 1.04–1.47). A high level of heterogeneity was also detected between these studies ($I^2=61\%$; $P<0.01$). The largest effect size was observed with respect to T2D (OR, 1.37; 95% CI, 1.10–1.72). Significant heterogeneity was detected ($I^2=91\%$; $P<0.01$) among the 7 studies meta-analyzed using a random effects model. The 1 study not included into the quantitative meta-analysis reported an association between a decrease in LTL and T2D (OR, 1.24; 95% CI, 1.09–1.42).\textsuperscript{23}

**Secondary Outcomes: CAD, CVD Death, MACE Composite**
A significant association between per-SD decrease in LTL and CAD was not observed when 7 studies assessing the outcome were pooled using a fixed effect model (OR, 1.03; 95% CI, 0.98–1.08; $I^2=41\%$). However, positive associations between per-SD decrease in LTL and the CVD death and MACE outcomes were identified. With respect to CVD death, 6 studies were combined using a fixed effect model to

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### Table 2. Characteristics of Included Studies Assessing the Association Between LTL and Myocardial Infarction

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up, y</th>
<th>Events/nonevents, n</th>
<th>Hospital vs Population Based</th>
<th>Average Age, y</th>
<th>Comorbidity</th>
<th>Ethnicity</th>
<th>LTL Assay</th>
<th>CV, %</th>
<th>NOS Quality Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brouillette et al\textsuperscript{24}</td>
<td>Case–control</td>
<td>...</td>
<td>203/180</td>
<td>Hospital</td>
<td>47</td>
<td>...</td>
<td>White</td>
<td>SB</td>
<td>3.3</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Zee et al\textsuperscript{25}</td>
<td>Case–control</td>
<td>...</td>
<td>337/337‡</td>
<td>Population</td>
<td>60</td>
<td>...</td>
<td>Mixed</td>
<td>qPCR</td>
<td>5.0</td>
<td>3 2 3</td>
</tr>
<tr>
<td>Fitzpatrick et al\textsuperscript{22}</td>
<td>Cohort</td>
<td>7.0</td>
<td>36/352</td>
<td>Population</td>
<td>74</td>
<td>...</td>
<td>Mixed</td>
<td>SB</td>
<td>1.5</td>
<td>2 2 2</td>
</tr>
<tr>
<td>Fyhrquist et al\textsuperscript{13,24}</td>
<td>Cohort</td>
<td>&gt;4.0</td>
<td>69/1202</td>
<td>Hospital</td>
<td>64</td>
<td>LVH</td>
<td>Mixed</td>
<td>SB</td>
<td>3.7</td>
<td>2 2 3</td>
</tr>
<tr>
<td>Weischer et al\textsuperscript{26}</td>
<td>Cohort</td>
<td>17,§ 6‖</td>
<td>939/18355</td>
<td>Population</td>
<td>58</td>
<td>...</td>
<td>White</td>
<td>qPCR</td>
<td>9.0</td>
<td>3 2 3</td>
</tr>
<tr>
<td>Willeit et al\textsuperscript{14}</td>
<td>Cohort</td>
<td>4.4</td>
<td>43/757</td>
<td>Population</td>
<td>63</td>
<td>...</td>
<td>White</td>
<td>qPCR</td>
<td>2.4</td>
<td>2 2 2</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation; LTL, leukocyte telomere length; LVH, left ventricular hypertrophy; NOS, Newcastle–Ottawa Scale; qPCR, quantitative polymerase chain reaction; and SB, southern blot.

*CV=SD/mean of replicates run at different time points.
†Newcastle–Ottawa Scale: C, comparability (scored out of 2); E/O, exposure/outcome (scored out of 3); and S, selection (scored out of 4).
‡Men only.
§Copenhagen City Heart Study; ‖Copenhagen General Population Study.

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### Table 3. Characteristics of Included Studies Assessing the Association Between LTL and Type 2 Diabetes Mellitus

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up, y</th>
<th>Events/nonevents, n</th>
<th>Hospital vs Population Based</th>
<th>Average Age, y</th>
<th>Comorbidity</th>
<th>Ethnicity</th>
<th>LTL Assay</th>
<th>CV, %</th>
<th>NOS Quality Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivieri et al\textsuperscript{27}</td>
<td>Case–control</td>
<td>...</td>
<td>103/104</td>
<td>Population</td>
<td>69</td>
<td>...</td>
<td>White</td>
<td>qPCR</td>
<td>6.0</td>
<td>3 2 2</td>
</tr>
<tr>
<td>Salpea et al\textsuperscript{28}</td>
<td>Case–control</td>
<td>...</td>
<td>569/448</td>
<td>Population</td>
<td>59</td>
<td>...</td>
<td>Mixed</td>
<td>qPCR</td>
<td>5.6</td>
<td>2 1 2</td>
</tr>
<tr>
<td>Shen et al\textsuperscript{29}</td>
<td>Case–control</td>
<td>...</td>
<td>1936/2080</td>
<td>Population</td>
<td>65</td>
<td>...</td>
<td>Asian</td>
<td>qPCR</td>
<td>2.0</td>
<td>4 2 2</td>
</tr>
<tr>
<td>You et al\textsuperscript{30}</td>
<td>Case–control</td>
<td>...</td>
<td>1668/2361†</td>
<td>Population</td>
<td>62</td>
<td>...</td>
<td>Hispanic</td>
<td>qPCR</td>
<td>5.7</td>
<td>3 2 2</td>
</tr>
<tr>
<td>Zee et al\textsuperscript{31}</td>
<td>Case–control</td>
<td>...</td>
<td>434/424</td>
<td>Population</td>
<td>56</td>
<td>...</td>
<td>Mixed</td>
<td>qPCR</td>
<td>5.0</td>
<td>4 2 3</td>
</tr>
<tr>
<td>Hovatta et al\textsuperscript{32}</td>
<td>Cohort</td>
<td>8.5</td>
<td>130/172</td>
<td>Hospital</td>
<td>55</td>
<td>IGT</td>
<td>White</td>
<td>qPCR</td>
<td>14</td>
<td>2 2 3</td>
</tr>
<tr>
<td>Zhao et al\textsuperscript{33}</td>
<td>Cohort</td>
<td>5.5</td>
<td>292/2036</td>
<td>Population</td>
<td>40</td>
<td>...</td>
<td>Native American</td>
<td>qPCR</td>
<td>6.9</td>
<td>3 2 2</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation; IGT, impaired glucose tolerance; LTL, leukocyte telomere length; NOS, Newcastle–Ottawa Scale; and qPCR, quantitative polymerase chain reaction.

*CV=SD/mean of replicates run at different time points.
†Newcastle–Ottawa Scale: C, comparability (scored out of 2); E/O, exposure/outcome (scored out of 3); and S, selection (scored out of 4).
‡Women only.
obtain a pooled OR that reached significance (OR, 1.11; 95% CI, 1.00–1.22; \( I^2 = 29\% \)). Three studies reported a MACE composite and when meta-analyzed using a fixed effect model a significant association was observed (OR, 1.14; 95% CI, 1.02–1.29). A high level of heterogeneity was present (\( I^2 = 64\% \)). See Figures I to III in the Data Supplement for corresponding forest plots.

Subgroup Analysis

Subgroup analysis by measurement technique, study type, study size, and ethnicity is presented for each primary outcome in Figure 3. Stratifying by qPCR or Southern blot explained some of the heterogeneity in the association between shortened LTL and stroke. \( I^2 \) decreased from 61% overall to 35% for studies using Southern blot and 58% for studies using qPCR (\( P = 0.03 \) for subgroup differences).

Stratifying by study design explained the high level of heterogeneity within the MI (\( I^2 = 85\% \)) was almost completely eliminated when stratifying studies by 0 to 499, 500 to 999, and >1000 participants (\( P = 0\% , 0\% , \) and 37%, respectively). The association with shortened LTL remained in the 2 smaller sized subgroups.

Stratifying by mean age explained some of the heterogeneity within the stroke meta-analysis. \( I^2 \) decreased to 2% for studies using participants with a mean age between 51 and 60 years and 38% for studies using participants with a mean age between 61 and 70 years (\( P = 0.01 \) for subgroup differences).

Significant subgroup differences were not observed when stratifying by ethnicity or study design.

Assessment of Publication Bias and Sensitivity Analysis

Publication bias was assessed for all outcomes through visually inspecting asymmetry in the funnel plots presented in Figure 4. The funnel plots for both MI and T2D demonstrated moderate asymmetry indicating publication bias. There was little evidence to suggest significant publication bias with respect to stroke and secondary outcomes (Figure

### Figure 2.

Forest plot of primary cardiometabolic outcomes. Results are presented for random effects models. CI indicates confidence interval; IV, inverse variance method; and LTL, leukocyte telomere length.

### Table 1

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Events</th>
<th>Non-Events</th>
<th>Weight</th>
<th>Odds Ratio, 95% CI</th>
<th>Odds Ratio IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ding(2) 2013</td>
<td>1309</td>
<td>1309</td>
<td>10.5%</td>
<td>1.34 [1.20, 1.49]</td>
<td></td>
</tr>
<tr>
<td>Ding(1) 2013</td>
<td>137</td>
<td>721</td>
<td>12.3%</td>
<td>1.04 [0.92, 1.22]</td>
<td></td>
</tr>
<tr>
<td>Fitzpatrick 2007</td>
<td>42</td>
<td>357</td>
<td>2.0%</td>
<td>2.79 [1.19, 6.54]</td>
<td></td>
</tr>
<tr>
<td>Fyhrquist 2011</td>
<td>43</td>
<td>1228</td>
<td>8.2%</td>
<td>1.56 [1.10, 2.21]</td>
<td></td>
</tr>
<tr>
<td>Jiang 2013</td>
<td>150</td>
<td>150</td>
<td>9.7%</td>
<td>1.00 [0.74, 1.35]</td>
<td></td>
</tr>
<tr>
<td>Schruck 2013</td>
<td>504</td>
<td>504</td>
<td>11.2%</td>
<td>1.01 [0.79, 1.31]</td>
<td></td>
</tr>
<tr>
<td>Willet 2010</td>
<td>46</td>
<td>754</td>
<td>8.9%</td>
<td>1.49 [1.08, 2.06]</td>
<td></td>
</tr>
<tr>
<td>Yang 2009</td>
<td></td>
<td>45</td>
<td>4.5%</td>
<td>1.69 [0.99, 2.89]</td>
<td></td>
</tr>
<tr>
<td>Zee(1) 2010</td>
<td>259</td>
<td>259</td>
<td>6.3%</td>
<td>1.05 [0.59, 1.84]</td>
<td></td>
</tr>
<tr>
<td>Zhang 2013</td>
<td>503</td>
<td>1801</td>
<td>10.3%</td>
<td>1.06 [0.95, 1.16]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>2993</td>
<td>7083</td>
<td>100.0%</td>
<td>1.21 [1.06, 1.37]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau^2 = 0.02, Chisq = 23.08, df = 9 (\( P = 0.008 \)); \( P = 61\% \)
Test for overall effect Z = 2.91 (\( P = 0.004 \))

<table>
<thead>
<tr>
<th>Myocardial Infarction</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Brouillette 2003</td>
<td>203</td>
<td>180</td>
<td>16.5%</td>
<td>1.56 [1.20, 2.03]</td>
<td></td>
</tr>
<tr>
<td>Fitzpatrick 2007</td>
<td>36</td>
<td>352</td>
<td>12.3%</td>
<td>1.30 [0.91, 1.87]</td>
<td></td>
</tr>
<tr>
<td>Fyhrquist 2011</td>
<td>60</td>
<td>1202</td>
<td>16.5%</td>
<td>0.90 [0.69, 1.17]</td>
<td></td>
</tr>
<tr>
<td>Welscher 2012</td>
<td>929</td>
<td>18356</td>
<td>25.2%</td>
<td>1.07 [0.99, 1.17]</td>
<td></td>
</tr>
<tr>
<td>Willet 2010</td>
<td>43</td>
<td>757</td>
<td>13.6%</td>
<td>1.41 [1.02, 1.99]</td>
<td></td>
</tr>
<tr>
<td>Zee(1) 2009</td>
<td>337</td>
<td>337</td>
<td>15.5%</td>
<td>1.45 [1.10, 1.91]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>1617</td>
<td>71183</td>
<td>100.0%</td>
<td>1.24 [1.04, 1.47]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau^2 = 0.03, Chisq = 15.66, df = 5 (\( P = 0.008 \)); \( P = 66\% \)
Test for overall effect Z = 2.43 (\( P = 0.02 \))
IV in the Data Supplement). Removing studies at high or moderate risk of bias according to the overall Newcastle–Ottawa Scale quality score did not significantly alter the associations in any of the cardiometabolic outcomes of interest. Similarly, removing studies not reporting a coefficient of variation (or reporting a coefficient of variation >10%) had no significant effect on any pooled ORs (Table XII in the Data Supplement).

**Discussion**

In this systematic review and meta-analysis of 27 observational studies, a constant positive association with per-SD decrease in LTL was observed across all primary cardiometabolic outcomes assessed. The main strength of this review lies in the fact that it is a pooled analysis of LTL and cardiometabolic outcomes, thus providing the greatest power to detect associations missed by smaller individual studies. Furthermore, the large number of outcomes assessed within this review allows for a more comprehensive evaluation of LTL as a general biomarker of aging.

MI is a consequence of interrupted blood flow to the heart subsequently leading to the death of cardiomyocytes. Likewise, stroke is characterized by the sudden loss of blood supply to the brain resulting in neuronal death. Both cardiometabolic outcomes are often caused by the formation of unstable atherosclerotic plaques over time within vascular tissue. It has been shown that plaques form as a product of impaired endothelial repair and vessel remodeling, high

**Figure 3.** Summary of subgroup analyses for primary outcomes. Results are presented for random effects models. CI indicates confidence interval; IV, inverse variance method; LTL, leukocyte telomere length; N/A, not applicable; and qPCR, quantitative polymerase chain reaction.
cell turnover, increased oxidative stress, and upregulation of inflammatory factors. Interestingly, these plaque formation processes have all been shown to be associated with decreased telomere length in vascular cells. When considered with the fact that LTL is highly correlated with vascular tissue telomere length, it is reasonable to expect shortened LTL in patients at risk of stroke or MI. Evidence from our meta-analysis aligns directly with this hypothesis as we have found that a per-SD decrease in LTL confers a higher risk for both stroke (OR, 1.21; 95% CI, 1.06–1.37) and MI (OR, 1.24; 95% CI, 1.04–1.47).

CVD-related death had the smallest effect size, despite all primary cardiometabolic outcomes demonstrating significant associations with a shortened LTL. A possible explanation for this is a lack of statistical power because of a relatively low number of events observed. In addition, the age of participants studied may have diminished the pooled estimate as it has been reported that LTL is a poor predictor of survival in elderly individuals (aged >75 years). Two studies included in the meta-analysis used populations with a mean age >75 years.

An interesting result was the significant association between a shortened LTL and the MACE composite outcome, suggesting that patients experiencing any events because of general cardiovascular aging had a shorter LTL. This effect has been observed and quantified in a study where it was shown that LTL in patients with CAD was similar to that of healthy controls who were 11 years older.

T2D is a metabolic disorder characterized by increased blood glucose levels because of pancreatic β-cell dysfunction in the context of increased insulin requirements. Because T2D is a strong predictor for CVD, it has been hypothesized that a common biological pathway based on tissue aging and senescence could potentially link the 2 diseases. Our findings of a significant relationship between a shortened LTL and T2D provide evidence for this hypothesis. Further support of this relationship can be seen in studies reporting cardiovascular events in diabetic patients. For instance, it has been shown that patients with T2D and MI have a shorter LTL when compared with T2D controls.

Paradoxically, CAD was the only outcome measured to not have a significant association with shortened LTL, although a consistent trend was observed (OR, 1.03; 95% CI, 0.98–1.08). Given our other findings, this result may imply that LTL is a potential marker of plaque rupture and thrombosis causing MI rather than the progression of atherosclerosis. However, there is substantial evidence for a biological relationship between shortened telomere length and atherosclerosis. Notably, a recent genome wide meta-analysis revealed an association between single-nucleotide polymorphisms associated with shortened LTL and an increased risk of CAD. This suggests a causal relationship between shortened telomere length and atheroma plaque build-up although MI was included in the definition of CAD. A likely reason for our findings for CAD is that several studies were excluded either because they did not meet our strict clinical definition of CAD (angina or nonfatal ischemic heart disease) or because we could not extract relevant effect estimates.

Heterogeneity was observed in some of the subgroup analyses; however, this was not consistent across all 3 primary outcomes. As compared with qPCR, the use of Southern blot was associated with a modestly stronger effect estimate for stroke, but not for MI. A plausible explanation is increased measurement error associated with qPCR biased the effect estimate toward the null, although sample size was also smaller in studies using Southern blot. A subgroup difference between study designs in the MI meta-analysis was also observed. Only the case–control subgroup remained significant (OR, 1.51; 95% CI, 1.25–1.82) after stratification suggesting that reverse causation or other biases might inflate risk estimates. Finally, study size was inversely correlated with strength of association for T2D and MI, indicating potential publication bias.

Limitations

Some limitations exist to the results presented in this meta-analysis. First, reporting of LTL as a variable differed between many studies and consequently statistical techniques were used to standardize reported effect measures to per-SD decrease. These statistical methods are most accurate for converting from LTL categorized as an ordinal variable (tertiles or quartiles) when there is a linear association with risk. Most studies included in this meta-analysis demonstrate the linearity of this association, but because of smaller sample sizes some studies deviate from it. Moreover, based on the assumption that LTL is a true biomarker for aging, the inclusion of effect measures adjusted for chronological age likely attenuates the strength of association with cardiometabolic outcomes. With the exception of 2, all studies included in this meta-analysis are adjusted for age and as such our findings are likely underestimates of underlying associations.

Finally, ethnic subgroup analysis was hindered because of lack of reporting of ethnicity-specific effect measures. Some studies included into the meta-analysis used multietnic populations and adjusted for this in their analysis but did not report ethnic-specific estimates. These authors were contacted...
for further information on ethnic group–specific effect measures, but no response was received. Although limited, we performed the subgroup analysis including a mixed group to represent studies reporting adjusted analysis for multietnic populations.

Conclusions

We present a systematic review and meta-analysis evaluating the use of LTL as a biomarker for aging through its association with age-related cardiometabolic outcomes. Despite a significant association between per-SD decrease in LTL and all primary outcomes measured, the results from this meta-analysis should be interpreted carefully as the observed heterogeneity is yet to be fully explained. Larger observational studies, with well-characterized patient populations and reliable LTL measurement techniques, are required to further explore sources of heterogeneity and ultimately validate the use of LTL as a marker for biological age.

Disclosures

None.

References


Telomeres are repetitive gene-poor regions that cap the ends of chromosomes and play a major role in preserving the stability of DNA. Decreasing in length as a result of increased oxidative stress and chronic inflammation within the cellular environment, telomeres are hypothesized to be a biological marker of aging and age-related diseases. Cardiometabolic outcomes such as cardiovascular disease and type 2 diabetes mellitus are clearly related to age, yet their association with leukocyte telomere length (LTL) is inconsistent within the current literature. It is critical to clarify these associations because LTL is suggested to be a potential clinical biomarker for cardiometabolic risk assessment. Through our systematic review and meta-analysis, we demonstrate that there is indeed an association between a 1 SD decrease in LTL and stroke (odds ratio, 1.21; 95% confidence interval, 1.06–1.37), myocardial infarction (odds ratio, 1.24; 95% confidence interval, 1.04–1.47), and type 2 diabetes mellitus (odds ratio, 1.37; 95% confidence interval, 1.10–1.72). Our reported associations, however, all have significant heterogeneity ($I^2=61\%$, $68\%$, and $91\%$, respectively). These results have important clinical implications as they provide evidence that LTL measurements must be interpreted cautiously. The high levels of heterogeneity need to be fully explained before precise cardiometabolic risk estimates can be provided to patients. Notably, the effect of patient ethnicity on LTL associations is yet to be fully explored and may account for the high variability in effect estimates.

**Clinical Perspective**

**CLINICAL PERSPECTIVE**

Telomeres are repetitive gene-poor regions that cap the ends of chromosomes and play a major role in preserving the stability of DNA. Decreasing in length as a result of increased oxidative stress and chronic inflammation within the cellular environment, telomeres are hypothesized to be a biological marker of aging and age-related diseases. Cardiometabolic outcomes such as cardiovascular disease and type 2 diabetes mellitus are clearly related to age, yet their association with leukocyte telomere length (LTL) is inconsistent within the current literature. It is critical to clarify these associations because LTL is suggested to be a potential clinical biomarker for cardiometabolic risk assessment. Through our systematic review and meta-analysis, we demonstrate that there is indeed an association between a 1 SD decrease in LTL and stroke (odds ratio, 1.21; 95% confidence interval, 1.06–1.37), myocardial infarction (odds ratio, 1.24; 95% confidence interval, 1.04–1.47), and type 2 diabetes mellitus (odds ratio, 1.37; 95% confidence interval, 1.10–1.72). Our reported associations, however, all have significant heterogeneity ($I^2=61\%$, $68\%$, and $91\%$, respectively). These results have important clinical implications as they provide evidence that LTL measurements must be interpreted cautiously. The high levels of heterogeneity need to be fully explained before precise cardiometabolic risk estimates can be provided to patients. Notably, the effect of patient ethnicity on LTL associations is yet to be fully explored and may account for the high variability in effect estimates.
Association Between Shortened Leukocyte Telomere Length and Cardiometabolic Outcomes: Systematic Review and Meta-Analysis
Matthew J.J. D'Mello, Stephanie A. Ross, Matthias Briel, Sonia S. Anand, Hertzel Gerstein and Guillaume Paré

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http://circgenetics.ahajournals.org/content/8/1/82

Data Supplement (unedited) at:
http://circgenetics.ahajournals.org/content/suppl/2014/11/18/CIRCGENETICS.113.000485.DC1
SUPPLEMENTAL METHODS

Statistical Conversion Technique

ORs (and HRs) that used LTL as an ordinal variable and compared incidence of event in the shortest versus longest group were converted to per-SD decrease in LTL. To accomplish this we first obtained the beta value by taking the log_e of the OR. Assuming a normal distribution, we determined the standard deviation of the data. We then divided the beta by this standard deviation and exponentiated the result to obtain the OR per-SD decrease in LTL.
## SUPPLEMENTARY TABLES

### Supplemental Table 1. Search Strategy for EMBASE (1980 - present) through OVID interface. Last conducted September 9th 2013

<table>
<thead>
<tr>
<th>#</th>
<th>Searches</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>telomere.mp. or exp telomere/ or exp telomere shortening/</td>
<td>20265</td>
</tr>
<tr>
<td>2</td>
<td>stroke.mp. or exp cerebrovascular accident/</td>
<td>262193</td>
</tr>
<tr>
<td>3</td>
<td>myocardial infarction.mp. or exp heart infarction/</td>
<td>283573</td>
</tr>
<tr>
<td>4</td>
<td>diabetes.mp. or exp diabetes mellitus/</td>
<td>638259</td>
</tr>
<tr>
<td>5</td>
<td>cardiovascular disease.mp. or exp cardiovascular disease/</td>
<td>2811015</td>
</tr>
<tr>
<td>6</td>
<td>exp cardiovascular mortality/ or exp mortality/ or mortality.mp.</td>
<td>840645</td>
</tr>
<tr>
<td>7</td>
<td>exp death/ or death.mp, or exp heart death/</td>
<td>835628</td>
</tr>
<tr>
<td>8</td>
<td>2 or 3 or 4 or 5 or 6 or 7</td>
<td>4293436</td>
</tr>
<tr>
<td>9</td>
<td>1 and 8</td>
<td>2522</td>
</tr>
<tr>
<td>10</td>
<td>Limit 9 to human</td>
<td>1999</td>
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</table>
**Supplemental Table 2.** Search Strategy for MEDLINE (1946 – present) through OVID interface. Last conducted September 9th 2013

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<th>Searches</th>
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<tr>
<td>1</td>
<td>telomere.mp. or exp telomere/ or exp telomere shortening/</td>
<td>16968</td>
</tr>
<tr>
<td>2</td>
<td>exp stroke, lacunar/ or exp stroke/ or stroke.mp.</td>
<td>185795</td>
</tr>
<tr>
<td>3</td>
<td>diabetes.mp. or exp diabetes mellitus, type 2/ or exp diabetes</td>
<td>418147</td>
</tr>
<tr>
<td>4</td>
<td>cardiovascular disease.mp. or exp cardiovascular diseases/</td>
<td>1884627</td>
</tr>
<tr>
<td>5</td>
<td>cerebrovascular.mp. or exp cerebrovascular disorders/</td>
<td>317226</td>
</tr>
<tr>
<td>6</td>
<td>myocardial infarction.mp. or exp myocardial infarction/</td>
<td>188203</td>
</tr>
<tr>
<td>7</td>
<td>mortality.mp. or exp mortality/</td>
<td>626231</td>
</tr>
<tr>
<td>8</td>
<td>exp death/ or death.mp.</td>
<td>570732</td>
</tr>
<tr>
<td>9</td>
<td>2 or 3 or 4 or 5 or 6 or 7 or 8</td>
<td>3051388</td>
</tr>
<tr>
<td>10</td>
<td>1 and 9</td>
<td>1627</td>
</tr>
<tr>
<td>11</td>
<td>limit 10 to humans</td>
<td>1383</td>
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</tbody>
</table>
**Supplemental Table 3.** Characteristics of included studies assessing the association between LTL and cardiovascular death.

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up (years)</th>
<th>Events/Non-Events, n</th>
<th>Hospital vs population based</th>
<th>Average Age (years)</th>
<th>Co-morbidity</th>
<th>Ethnicity</th>
<th>LTL assay</th>
<th>CV* (%)</th>
<th>NOS Quality Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eppel et al., 2009¹</td>
<td>Cohort</td>
<td>12</td>
<td>53/182</td>
<td>Population</td>
<td>74</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>NR</td>
<td>3 2 1</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2011²</td>
<td>Cohort</td>
<td>6.1</td>
<td>103/1031</td>
<td>Population</td>
<td>74</td>
<td>-</td>
<td>Mixed</td>
<td>S.Blot</td>
<td>1.5</td>
<td>4 2 3</td>
</tr>
<tr>
<td>Houben et al., 2011³</td>
<td>Cohort</td>
<td>7.0</td>
<td>17/186²</td>
<td>Population</td>
<td>78</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>NR</td>
<td>3 2 2</td>
</tr>
<tr>
<td>Martin-Ruiz et al., 2005⁴</td>
<td>Cohort</td>
<td>3.0</td>
<td>249/977</td>
<td>Population</td>
<td>90</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>2.1</td>
<td>3 0 2</td>
</tr>
<tr>
<td>Njajou et al., 2009⁵</td>
<td>Cohort</td>
<td>10</td>
<td>69/714</td>
<td>Population</td>
<td>74</td>
<td>-</td>
<td>Mixed</td>
<td>qPCR</td>
<td>NR</td>
<td>3 0 3</td>
</tr>
<tr>
<td>Willeit et al., 2010⁶</td>
<td>Cohort</td>
<td>4.4</td>
<td>45/755</td>
<td>Population</td>
<td>63</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>2.4</td>
<td>2 2 2</td>
</tr>
</tbody>
</table>
NR = Not Reported; S.Blot = Southern Blot

*CV= standard deviation / mean of replicates run at different time points.

† Newcastle Ottawa Scale: S = Selection (scored out of 4), C= Comparability (scored out of 2), E/O= Exposure/Outcome (scored out of 3)

‡ Males Only
**Supplemental Table 4.** Characteristics of included studies assessing the association between LTL and the MACE.

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up (years)</th>
<th>Events/Non-events, n</th>
<th>Hospital vs population based</th>
<th>Average Age (years)</th>
<th>Co-morbidity</th>
<th>Ethnicity</th>
<th>LTL assay</th>
<th>CV* (%)</th>
<th>NOS Quality Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farzaneh-Far et al., 2008</td>
<td>Cohort</td>
<td>4.4</td>
<td>118/662</td>
<td>Hospital</td>
<td>68</td>
<td>Outpatients</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>2.4</td>
<td>3 2 2 2</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011</td>
<td>Cohort</td>
<td>&gt;4.0</td>
<td>134/1137</td>
<td>Hospital</td>
<td>64</td>
<td>Hypertensive, ventricular hypertrophy</td>
<td>Caucasian</td>
<td>S.Blot</td>
<td>3.7</td>
<td>2 2 3</td>
</tr>
<tr>
<td>Willeit et al., 2010</td>
<td>Cohort</td>
<td>4.4</td>
<td>88/712</td>
<td>Population</td>
<td>63</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>2.4</td>
<td>2 2 2 2</td>
</tr>
</tbody>
</table>

NR = Not Reported; S.Blot = Southern Blot

*CV= standard deviation / mean of replicates run at different time points.

† Newcastle Ottawa Scale: S = Selection (scored out of 4), C= Comparability (scored out of 2), E/O= Exposure/Outcome (scored out of 3)
### Supplemental Table 5. Characteristics of included studies assessing the association between LTL and CAD.

<table>
<thead>
<tr>
<th>Source</th>
<th>Study Design</th>
<th>Follow-Up (years)</th>
<th>Events/Non-Events, n</th>
<th>Hospital vs population based</th>
<th>Average Age (years)</th>
<th>Co-morbidity</th>
<th>Ethnicity</th>
<th>LTL assay</th>
<th>CV* (%)</th>
<th>NOS Quality Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ding et al., 2012⁹</td>
<td>Case-Control</td>
<td>-</td>
<td>71/787</td>
<td>Population</td>
<td>66</td>
<td>-</td>
<td>Asian</td>
<td>qPCR</td>
<td>1.3</td>
<td>4 2 3</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2007¹⁰</td>
<td>Cohort</td>
<td>6.1</td>
<td>52/293</td>
<td>Population</td>
<td>74</td>
<td>-</td>
<td>Mixed</td>
<td>S.Blot</td>
<td>1.5</td>
<td>4 2 3</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011⁸</td>
<td>Cohort</td>
<td>&gt;4.0</td>
<td>69/1202</td>
<td>Hospital</td>
<td>64</td>
<td>ventricular hypertrophy</td>
<td>Mixed</td>
<td>S.Blot</td>
<td>3.7</td>
<td>2 2 3</td>
</tr>
<tr>
<td>Weisher et al., 2005¹¹</td>
<td>Cohort</td>
<td>17³, 6¹</td>
<td>2023/16723</td>
<td>Population</td>
<td>58</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>9.0</td>
<td>3 2 3</td>
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<tr>
<td>Willeit et al., 2009⁶</td>
<td>Cohort</td>
<td>4.4</td>
<td>33/767</td>
<td>Population</td>
<td>63</td>
<td>-</td>
<td>Caucasian</td>
<td>qPCR</td>
<td>2.4</td>
<td>2 2 2 2</td>
</tr>
<tr>
<td>Study</td>
<td>Cohort</td>
<td>Population</td>
<td>Hypertensive patients</td>
<td>Ethnicity</td>
<td>Method</td>
<td>Mean</td>
<td>CV</td>
<td>Score</td>
<td></td>
<td></td>
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<td>------</td>
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<td>--------</td>
<td></td>
<td></td>
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<tr>
<td>Yang et al., 2009</td>
<td>5.0</td>
<td>47/364</td>
<td>Asian qPCR</td>
<td>6.8</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ye et al., 2013</td>
<td>8.7</td>
<td>153/1778</td>
<td>Mixed qPCR</td>
<td>5-8</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NR = Not Reported; S.Blot = Southern Blot

*CV = standard deviation / mean of replicates run at different time points.

† Newcastle Ottawa Scale: S = Selection (scored out of 4), C = Comparability (scored out of 2), E/O = Exposure/Outcome (scored out of 3)
**Supplemental Table 6.** Adjustments reported for included studies assessing stroke

<table>
<thead>
<tr>
<th>Source</th>
<th>Reported Analysis Adjustment</th>
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<td>Ding et al., 2012</td>
<td>Age, sex, BMI, hypertension, diabetes, hyperlipidemia, smoking status</td>
</tr>
<tr>
<td>Jiang et al., 2013</td>
<td>Age, sex, hypertension, recent social pressures, HDL</td>
</tr>
<tr>
<td>Zee et al., 2010</td>
<td>Age, smoking status, time of follow up, randomization treatment group, BMI, hypertension, diabetes, hyperlipidemia</td>
</tr>
<tr>
<td>Zhang et al., 2013</td>
<td>Age, gender, BMI, systolic/diastolic BP, fasting glucose, triacylglycerol, total cholesterol, HDL/LDL, smoking status, alcohol intake, diabetes, history of hypertension, previous CHD, family history of stroke</td>
</tr>
<tr>
<td>Schurks et al., 2013</td>
<td>Age, smoking, postmenopausal status, post-menopausal hormone use, Elevated cholesterol, hypertension, diabetes, CHD, alcohol consumption, aspirin use, BMI, physical activity, total cholesterol/HDL ratio, HbA1c, healthy dietary score</td>
</tr>
<tr>
<td>Ding et al., 2012</td>
<td>Age, sex, BMI, hypertension, diabetes, hyperlipidemia, smoking status</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2007</td>
<td>Age, sex, race</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011</td>
<td>Age, sex</td>
</tr>
<tr>
<td>Willeit et al., 2010</td>
<td>Age, sex, previous stroke, hypertension, pack-years of smoking, ferritin, high-sensitivity C-reactive protein, lipoprotein(a), LDL, HDL, physical activity, diabetes mellitus, alcohol consumption</td>
</tr>
<tr>
<td>Yang et al., 2009</td>
<td>Age, gender, hypertension</td>
</tr>
</tbody>
</table>
### Supplemental Table 7. Adjustments reported for included studies assessing myocardial infarction

<table>
<thead>
<tr>
<th>Source</th>
<th>Reported Analysis Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brouilette et al., 2003¹⁹</td>
<td>Age, sex, smoking status</td>
</tr>
<tr>
<td>Zee et al., 2009²⁰</td>
<td>Age, smoking status, follow-up, treatment group, BMI, hypertension, diabetes, hyperlipidemia</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2007¹⁸</td>
<td>Age, sex, race</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011⁸</td>
<td>Age, sex</td>
</tr>
<tr>
<td>Weischer et al., 2012¹¹</td>
<td>Age, gender, study, cholesterol, triglycerides, high-density lipoprotein cholesterol, c-reactive protein, use of lipid lowering therapy, BMI, hypertension, diabetes, smoking, heavy alcohol intake, physical inactivity</td>
</tr>
<tr>
<td>Willeit et al., 2012⁹</td>
<td>Age, sex, previous MI, hypertension, pack-years of smoking, ferritin, high-sensitivity C-reactive protein, lipoprotein(a), LDL, HDL, physical activity, diabetes mellitus, alcohol consumption</td>
</tr>
</tbody>
</table>
**Supplemental Table 8.** Adjustments reported for included studies assessing cardiovascular death

<table>
<thead>
<tr>
<th>Source</th>
<th>Reported Analysis Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epel et al., 2009¹</td>
<td>Age, Sex</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2011²</td>
<td>Age, sex, African-American race, hypertension, diabetes (ADA), smoking status, history of CAD, stroke, CHF, c-reactive protein, interleukin-6</td>
</tr>
<tr>
<td>Houben et al., 2011³</td>
<td>Age, smoking status, alcohol use, body mass index, education, marital status, physical activity, chronic diseases</td>
</tr>
<tr>
<td>Martin-Ruiz et al., 2005⁴</td>
<td>Age censored</td>
</tr>
<tr>
<td>Njajou et al., 2009⁵</td>
<td>None</td>
</tr>
<tr>
<td>Willeit et al., 2010⁶</td>
<td>Age, sex, hypertension, pack-years of smoking, ferritin, high-sensitivity C-reactive protein, lipoprotein(a), LDL, HDL, physical activity, diabetes mellitus, alcohol consumption</td>
</tr>
<tr>
<td>Source</td>
<td>Reported Analysis Adjustment</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Monikarage et al., 2012</td>
<td>Age, sex, waist circumference, adiponectin, telomere length, TBARS, insulin resistance</td>
</tr>
<tr>
<td>Olivieri et al., 2009</td>
<td>Age, sex, glucose, HbA1C waist-to hip ratio</td>
</tr>
<tr>
<td>Salpea et al., 2010</td>
<td>Age</td>
</tr>
<tr>
<td>Shen et al., 2012</td>
<td>Age, sex, BMI, smoking, and drinking</td>
</tr>
<tr>
<td>You et al., 2012</td>
<td>Age, ethnicity, date of blood collection, clinical center, BMI physical activity, hormone therapy, alcohol consumption, smoking</td>
</tr>
<tr>
<td>Zee et al., 2010</td>
<td>Age, smoking status, BMI, menopausal status, sex</td>
</tr>
<tr>
<td>Hovatta et al., 2012</td>
<td>Age, sex, randomization group</td>
</tr>
<tr>
<td>Zhao et al., 2013</td>
<td>Age, sex, age-squared, BMI, fasting glucose, and triglyceride level at baseline</td>
</tr>
</tbody>
</table>
**Supplemental Table 10.** Adjustments reported for included studies assessing MACE composite outcome

<table>
<thead>
<tr>
<th>Source</th>
<th>Reported Analysis Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farzaneh-Far et al., 2008⁷</td>
<td>Age, gender, ethnicity, LDL Cholesterol, HDL Cholesterol, Systolic Blood Pressure, Diastolic Blood Pressure, BMI, Stroke, Diabetes, CHF, Log CRP, LVEF, Diastolic dysfunction</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011⁸</td>
<td>Age, sex</td>
</tr>
<tr>
<td>Willeit et al., 2010⁵</td>
<td>Age, sex, previous CVD, hypertension, pack-years of smoking, ferritin, high sensitivity C-reactive protein, lipoprotein(a), and low-/high-density lipoprotein cholesterol levels, physical activity, diabetes mellitus, alcohol consumption.</td>
</tr>
</tbody>
</table>
**Supplemental Table 11.** Adjustments reported for included studies assessing coronary artery disease composite outcome

<table>
<thead>
<tr>
<th>Source</th>
<th>Reported Analysis Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ding et al., 2012(^9)</td>
<td>Age, sex, BMI, hypertension, diabetes, hyperlipidemia, smoking status</td>
</tr>
<tr>
<td>Fitzpatrick et al., 2007(^1)</td>
<td>Age, race, and gender</td>
</tr>
<tr>
<td>Fyhrquist et al., 2011(^6)</td>
<td>Age, sex</td>
</tr>
<tr>
<td>Weisher et al., 2012(^)</td>
<td>Age, gender, study, cholesterol, triglycerides, high-density lipoprotein cholesterol, c-reactive protein, use of lipid lowering therapy, BMI, hypertension, diabetes, smoking, heavy alcohol intake, physical inactivity</td>
</tr>
<tr>
<td>Willeit et al., 2009(^6)</td>
<td>Age, sex, previous MI, hypertension, pack-years of smoking, ferritin, high-sensitivity</td>
</tr>
<tr>
<td>Yang et al., 2009(^1)</td>
<td>C-reactive protein, lipoprotein(a), LDL, HDL, physical activity, diabetes mellitus, alcohol consumption</td>
</tr>
<tr>
<td>Ye et al., 2013(^1)</td>
<td>Age, sex, hypertension</td>
</tr>
<tr>
<td></td>
<td>Age, sex, BMI, Framingham risk score, use of lipid-lowering medications, physical activity, ln(CRP), ln (II-6) and ln (sICAM-1)</td>
</tr>
</tbody>
</table>
**Supplemental Table 12. Sensitivity analysis.**

<table>
<thead>
<tr>
<th></th>
<th>Stroke</th>
<th>MI</th>
<th>T2D</th>
<th>CVD related death</th>
<th>MACE</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>1.21 [1.06, 1.37]</td>
<td>1.24 [1.04, 1.47]</td>
<td>1.37 [1.10, 1.72]</td>
<td>1.11 [1.00, 1.22]</td>
<td>1.14 [1.02, 1.29]</td>
<td>1.03 [0.98-2.08]</td>
</tr>
<tr>
<td>Remove High CV</td>
<td>1.29 [1.19, 1.40]</td>
<td>1.29 [1.13, 1.47]</td>
<td>1.28 [1.21, 1.36]</td>
<td>1.11 [0.97, 1.26]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Remove Studies at Risk of Bias</td>
<td>-</td>
<td>-</td>
<td>1.15 [1.09, 1.21]</td>
<td>1.15 [1.04, 1.26]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Remove Studies with Comorbidities</td>
<td>1.18 [1.02, 1.37]</td>
<td>1.32 [1.09, 1.59]</td>
<td>1.35 [1.07, 1.69]</td>
<td>-</td>
<td>1.69 [1.20, 2.39]</td>
<td>1.02 [0.97-2.07]</td>
</tr>
</tbody>
</table>

Blank spaces indicate no change in original effect estimate.
SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1. Forest plot of studies assessing LTL and a CAD composite outcome. Results are reported based on fixed effect model.

Supplemental Figure 2. Forest plot of studies assessing LTL and CVD death. Results are reported based on the fixed effect model.

Supplemental Figure 3. Forest plot of studies assessing LTL and a MACE composite outcome. Results are reported based on fixed effect model.

Supplemental Figure 4. Funnel plot depicting the level of publication bias within the (a) CAD, (b) CVD death, and (c) MACE outcomes
SUPPLEMENTARY FIGURES

Supplemental Figure 1.

Supplemental Figure 2.
Supplemental Figure 3.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Odds Ratio)</th>
<th>SE</th>
<th>Events Total</th>
<th>Non-Events Total</th>
<th>Odds Ratio IV, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farzaneh-Far 2008</td>
<td>0.09531</td>
<td>0.112712</td>
<td>118</td>
<td>662</td>
<td>29.4% 1.10 [0.88, 1.37]</td>
</tr>
<tr>
<td>Fyhrquist 2011</td>
<td>0.075107</td>
<td>0.079823</td>
<td>134</td>
<td>1137</td>
<td>58.6% 1.08 [0.92, 1.26]</td>
</tr>
<tr>
<td>Willeit 2010</td>
<td>0.524137</td>
<td>0.176503</td>
<td>88</td>
<td>712</td>
<td>12.0% 1.69 [1.20, 2.39]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td></td>
<td></td>
<td>340</td>
<td>2511</td>
<td>100.0% 1.14 [1.02, 1.29]</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 5.55, df = 2 (P = 0.06); I² = 64%
Test for overall effect: Z = 2.21 (P = 0.03)
Supplemental Figure 4

(a) 

(b) 

(c)
SUPPLEMENTARY REFERENCES


