The greatest obstacle to discovery is not ignorance—it is the illusion of knowledge.

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In their meta-analysis of 27 published studies, D’Mello et al show that shortened leukocyte telomere length (LTL) is associated with 3 primary end points: myocardial infarction, stroke, and type 2 diabetes mellitus. The meta-analysis and its findings raise several fundamental issues that are worth considering. After discussing the major findings and limitations, we focus on 3 central themes: the reliability of LTL measurements, the biological meaning of the association of LTL with cardiometabolic outcomes, and future directions of telomere research with respect to cardiovascular disease (CVD) and longevity in humans.

The underlying reason for meta-analysis is usually lack of consensus across studies. This clearly applies to the field of telomere epidemiology, which is awash with conflicting findings not only in the context of CVD but also cancer and longevity. The meta-analysis of D’Mello et al shows a significant association of LTL with each of the 3 primary end points, but the test for heterogeneity across the studies was significant for each disease. The authors suggest that ethnicity or perhaps demography, which is often associated with ethnicity, might explain some disagreements in findings across populations (and studies). Notably, however, 26 of the 27 studies analyzed across the 3 primary disease end points had odds ratios >1, indicating that the observed heterogeneity resulted from varying magnitudes of a positive association with disease and not from studies showing inverse associations. This observation lends further support to the conclusions by D’Mello et al of significant associations of short LTL with each of the 3 diseases. The findings are generally consistent with those of a recent systematic review and meta-analysis by Haycock et al that included 24 studies, many overlapping with those of D’Mello et al, although Haycock et al were less certain as to the association with cerebrovascular disease. The 2 studies differed in their definitions of coronary heart disease (CHD) and in their treatment of LTL—D’Mello as a continuous variable and Haycock as a categorical variable.

The subgroup analysis in Figure 3 of the D’Mello paper suggests that the racial or ethnic group with the highest prevalence of the specific disease showed the smallest association with LTL (Asians for stroke, Hispanics and African Americans for diabetes mellitus). It is unclear why this would be so, unless the higher prevalence of disease in these groups was caused by influential ethnic-related determinants independent of the basic biological and heritable influences of telomere length. The authors were not able to get further data on race and ethnic group from the contributing studies to adequately address these important potentially confounding variables. The number of studies available in some of the racial and ethnic groups was simply too few to draw reliable conclusions as to their influence on the heterogeneity of the estimated disease risks. Also, the inverse association seen in the meta-analysis between sample size and odds ratios estimates (ie, the smaller the study, the more likely the odds ratios estimates were to be elevated) is consistent with the likely publication bias suggested by the authors. Smaller studies with null results are probably unpublished as demonstrated by both Haycock et al and D’Mello et al.

Reliability of LTL Measurements

The reliability of LTL measurements and sample size are closely linked with respect to findings and conclusions because less reliable LTL measurements require larger samples to test associations and hypotheses. With an interassay coefficient of variation =4x higher for qPCR than for Southern blot, as shown by impartial comparisons in leading laboratories, the method of TL measurement becomes particularly important for small studies. This matter has been raised previously on multiple occasions. The meta-analysis by Haycock et al is consistent with this inference in that associations were weaker for qPCR-based studies than for other methods, although the underpowered tests for heterogeneity were not significant. Similarly, the subgroup analysis by D’Mello et al was underpowered to adequately evaluate likely heterogeneity because of methodology, lacking in the number of studies using Southern blots.

Atherosclerosis is probably the common denominator explaining the associations of LTL with CHD and occlusive stroke, which are principal manifestations of atherosclerosis, and with type 2 diabetes mellitus. D’Mello et al (and we) were
thus puzzled that what they termed coronary artery disease (a secondary outcome which they defined as angina and nonfatal ischemic heart disease) was not found to be significantly associated with LTL, a finding that might also relate to reliability of LTL measurements and sample size, as well as to the less definitive diagnosis. Similarly, no significant association was found for CVD mortality or a composite of events that included myocardial infarction, CVD death, and stroke. The single CHD definition used by Haycock (nonfatal myocardial infarction, CHD death, or coronary revascularization) may be more comprehensive as a primary outcome. However, further support for an association comes from the consistency in the association of LTL with CHD as defined by Haycock et al and myocardial infarction as per D’Mello et al.

Clarification of the LTL relationship with coronary artery disease (a term which might preferably be reserved for the underlying pathophysiological process, irrespective of clinical manifestation), may be obtained by looking at the coronary artery calcification (CAC) associations with LTL. CAC is perhaps currently the most useful noninvasive index of the burden of coronary atherosclerosis. Persons with no evidence of CAC or low CAC scores have minimal near-term risk of incurring myocardial infarction. Three studies, which were not included in the meta-analysis by D’Mello et al or Haycock et al, examined the association of LTL with CAC. Two of the studies (sample sizes 325 and 129) used the qPCR technique; one reported an inverse association of CAC with LTL, whereas the other did not detect such an association.5,6 The third study, which used the more accurate Southern blot method, observed an inverse association of CAC with LTL based on 250 subjects.7 Studies based on large sample sizes are clearly needed to establish with confidence that a short LTL is associated with increased asymptomatic atherosclerotic burden in the coronary circulation.

The statistical power considerations that arise from small sample size, especially when using less precise measurements of LTL, raise a troublesome question: How many conclusions about LTL associations or lack thereof with a host of human traits and diseases are based on solid evidence rather than representing statistical noise? The meta analysis papers by D’Mello et al and Haycock et al are steps in the right direction in this regard.

**Biological Meaning of the Association of LTL With Cardio-Metabolic Outcomes**

In the individual, LTL reflects telomere length in other somatic cells, including stem cells. It is commonplace to refer to LTL as a biomarker of aging, as do D’Mello et al. This concept has been the mainstay of telomere epidemiology, ignoring the accruing evidence that suggests otherwise. To illustrate the premise that LTL is an aging biomarker, D’Mello et al cite a paper by Brouilette et al showing that patients with CHD displayed an LTL that was shorter by 0.3 kilobases (kb) than LTL in controls. Using cross-sectional analysis, Brouilette et al estimated an average rate of 0.026 kb/year LTL shortening in adults; they further concluded, based on this rate, that LTL in patients with CHD was equivalent to that of healthy controls 11 years older. Stated differently, the authors inferred that the biological age of the patients with CHD was 11 years older than their chronological age. This deduction has been used by multiple investigators, including the authors of this editorial in earlier publications; it is highly flawed, however, because it does not account for the large interindividual variation in LTL at birth. Moreover, implicit in the use of the rate of LTL attrition during adulthood as an index of the pace of aging is the concept that a short LTL predominantly reflects the outcome of an accelerated LTL attrition occurring primarily during adult life. Once this misconception took root, it has been difficult to undo regardless of the evidence amassed against it. To further understand the origin of this misconception, let us briefly consider the research that led to the notion that LTL is a biomarker of human aging.

In cultured somatic cells, telomere length undergoes progressive attrition with successive cell replications.9 Accordingly, telomere shortening integrates the replicative history of these cells. In addition, oxidative stress apparently increases the loss of telomere repeats per replication of cultured cells.10 In vivo, telomere length in the hematopoietic system and other somatic tissues displays age-dependent attrition after birth. Aging and atherosclerosis reflect in part the cumulative burden of indolent oxidative stress and inflammation, which increases the turnover of leukocytes. Therefore, it seems reasonable to infer that individuals with a higher burden of oxidative stress and inflammation are biologically older, have more atherosclerosis, and display a shorter LTL than their peers. However, does extrapolation of telomere length dynamics in cultured somatic cells to the in vivo state make sense in defining LTL as a biomarker of aging? Moreover, to what extent does age-dependent LTL attrition influence the vast LTL variation across individuals of the same age? These questions have been addressed elsewhere,11 but here we would like to showcase a major shortcoming that renders LTL, which is highly heritable, a poor biomarker of human aging.

The range of LTL variation across newborns amounts to ≈4 kb (≈8–12 kb).12,13 Four kilobase is also the approximate range of LTL variation in adults, although telomere length in adults is shorter than in newborns. Little is known about LTL dynamics during the first 2 decades of life. However, longitudinal evaluations provide strong evidence that individuals who enter adulthood with short (or long) LTL display short (or long) LTL later in life.14,15 This means that LTL at birth and perhaps LTL dynamics during growth exert a lasting effect on LTL throughout the lifetime course. Yet based on considerations of LTL dynamics during adulthood (an average of 0.025 kb/yr LTL attrition), a newborn with an LTL of 8 kb would be biologically 160 years older than a newborn with an LTL of 12 kb.

Clearly, having short (or long) LTL antecedes by many decades the onset of aging-related disorders in most individuals. In this sense, LTL is hardly a biomarker of aging, although perhaps it might forecast as early as at birth the predilection to aging-related diseases in adulthood, which may also affect longevity. If LTL is a questionable biomarker of aging, what then are the underlying mechanisms that explain the shorter LTL in subjects with the cardio-metabolic outcomes? This question cuts to the core of the biological meaning of LTL because it prompts us to consider LTL not only as a manifestation, that is, a passive biomarker, of cardio-metabolic outcomes but also as one of the potential determinants.
A common approach to human disease and aging centers on injury, for example, oxidative stress and inflammation, with little attention devoted to repair. In the final analysis, however, aging and its health consequences reflect impaired homeostasis, defined by a growing imbalance between injury and repair. Relatively short telomeres in somatic tissues, as expressed in a shorter LTL, might then reflect diminished somatic reserves with respect to repair capacity.\textsuperscript{16,17} Simply put, we propose that a shorter telomere length entails a reduced ability of stem cells to engage in tissue repair, and a component of this tissue repair capacity, represented by telomere length, is determined at birth and during the early years of growth.

**Future Research Directions**

Age is a crucial determinant of CVD, given that the incidence of the disease, principally in the form of atherosclerosis, shows an exponential increase with age. As LTL undergoes age-dependent attrition, age is also a key factor in the relation between LTL and CVD. Accordingly, long and short LTLs and not having versus having CVD are traits defined in relative terms, based on comparisons with peers of the same age, sex, and ethnicity. What distinguishes individuals with a longer LTL versus those with a shorter LTL is not simply a lower lifetime risk of CVD in those with longer LTL. Rather, individuals with a longer LTL display a shift in the risk of CVD to an older age (Figure). Individuals might still get CVD because of age-related shortening of LTL, but only after they reach some threshold below which repair processes are compromised. Individuals born with shorter telomeres would cross this threshold sooner than those with longer telomeres. Age-related telomere shortening in adulthood would play a minor modulating role in the age of onset of disease compared with the much larger influence of telomere length at birth and telomere attrition early in life.

In our view, this concept compels us to rethink the traditional paradigm that links telomeres to CVD, and it is crucial for answering the question: where do we go from here? By now, we should know where not to go, namely, testing for associations of LTL with indices of inflammation and oxidative stress during adult life, based on cross-sectional studies. In our view, this approach will only marginally increase our insight into the LTL-CVD nexus. An increased burden of inflammation and oxidative stress during adulthood may indeed accelerate LTL attrition to some extent, but its effect is likely to be minor compared with the overwhelming influence on adult LTL of telomere length at birth and its attrition during growth. Moreover, most indices of inflammation and oxidative stress are snapshots of the metabolic status of the individual at the time of blood collection, whereas LTL reflects the entire life history of the individual from birth onward. Even if some meaningful information can be obtained about the effect of inflammation and oxidative stress on LTL dynamics, this can be validly gained only through longitudinal studies that follow LTL and the inflammation/oxidative status of the individual over many years.

Although telomere length can be readily measured in epidemiological settings, this is not the case for examining capacity for tissue repair. From the telomere perspective, increased repair capacity denotes increased proliferative potential, which might raise the risk for cancer. Perhaps one way to tackle this subject is through epidemiological studies exploring potential biological trade-offs of having short or long telomeres. For instance, variant genes, which are jointly associated with a short LTL, were used to develop a genetic risk score for CHD.\textsuperscript{18} The same variant genes, which are jointly associated with a long LTL, were also used to develop a genetic risk score for melanoma.\textsuperscript{19} These findings not only infer causality between telomere length (an intermediate phenotype) and disease, but also the possibility of a melanoma-atherosclerosis trade-off, which might also apply to some other cancers.

Finally and perhaps most importantly, in the quest for understanding the role of telomeres in CVD, other aging-related diseases, and longevity, most researchers have focused their work on middle age and elderly persons. However, to understand what makes telomeres long or short, it is our opinion that research should focus on factors that fashion telomere length at birth and telomere dynamics during early life.

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**References**


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