

# Telomeres and cardiovascular disease risk: an update 2013

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Leukocyte telomere length (LTL) has been regarded as a potential marker of biologic aging because it usually shortens in a predictable way with age. Recently, a growing interest in cardiovascular aging has led to a number of new epidemiologic studies investigating LTL in various disease conditions. Some methodological problems exist because there are different methods available to determine LTL, and standardization is much needed. For example, in the majority of studies, patients with early-onset coronary heart disease have been shown to have shorter LTL. In addition, patients with diabetes mellitus complications tend to have shorter LTL than control subjects. On the other hand, increased left ventricular hypertrophy or mass is associated with longer LTL, and studies investigating hypertension have reported both shorter and longer LTL than found in normotensive control subjects. There is, therefore, a need for longitudinal studies to elucidate these complicated relationships further, to provide estimations of telomere attrition rates, and to overcome analytical problems when only cross-sectional studies are used. The understanding of cardiovascular aging and telomere biology may open up new avenues for interventions, such as stem cell therapy or agents that could retard this aging process over and beyond conventional risk factor control. (*Translational Research* 2013;162:371–380)

**Abbreviations:** CAD = coronary artery disease; CHD = coronary heart disease; CVD = cardiovascular disease; EF = ejection fraction; FH = familiar hypercholesterolemia; HR = hazard ratio; IHD = ischemic heart disease; LTL = leukocyte telomere length; LVH = left ventricular hypertrophy; LVMI = left ventricular mass index; MI = myocardial infarction; q-PCR = quantitative polymerase chain reaction; RTL = relative telomere length; TBP = telomere binding protein

**T**elomeres form the end segment of the DNA helix and protect this end between mitosis. Ever since the Nobel Prize in 2009 was awarded to 3 American scientists for telomere research, the interest in telomere biology has grown substantially. One indication of this is the increasing number of publications found at PubMed, with currently (January 2013) almost 15,000 hits for “telomere” of which 420 are combined with “cardiovascular,” 357 with

“coronary,” 140 with “atherosclerosis,” and 169 with “diabetes.” This brief review focuses on the development of telomere research in relation to cardiovascular disease (CVD) and shows how complex the field is today.

## STRUCTURE AND FUNCTION OF TELOMERES

Telomeres are repetitive, noncoding DNA sequences (TTAGGG) located at both ends of each chromosome.

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The length of the telomeres in humans can reach up to 15,000 bp, ending with a single-strand overhang at the 3' end measuring 75 bp–200 bp. The telomere complex consists of several parts: the telomeres themselves, the regulating enzyme telomerase, and a few telomere binding proteins (TBP). Together with TBPs, in the shelterin complex, the telomeres form loop structures that are believed to protect the chromosome ends from fusing with each other, hence preventing possible oncogenic development.<sup>1</sup> The telomere loops also protect the chromosomal DNA from being recognized as damaged, reduce the risk of apoptosis, and stabilize the DNA complex. There is also the more recently described telomere-capping Cdc13-Stn1-Ten1 (CST)-complex that comprises 3 proteins—Cdc13 and Stn1, and related to the conserved telomere maintenance complex component 1 (Ten1) originally found in yeast, but also present in humans, and shown in genomewide association studies to participate in telomere maintenance.<sup>2</sup>

During each mitosis, the DNA polymerase replicates the DNA (including the telomeres), but is unable to replicate the last part of the lagging strand, resulting in progressive shortening of the chromosomes. This is called the end replication problem, and the DNA loss amounts to approximately 30 bp–150 bp per mitosis. Because of the non-sense DNA information on the telomeres, the cell can afford losing a few hundred base pairs during each replication without loss of coding DNA and possible oncogenic transformation. Ultimately, the ends of the telomeres become too close to the coding DNA region and the loop structures cannot be kept together. The protective function of the telomeres is lost, and this partially damaged DNA is recognized by tumor suppressors, for which the cell is either programmed for apoptosis or is irreversibly blocked during the G1 phase of mitosis, also termed replicative senescence. The senescent cells have other, more aging-related properties than dividing cells, express different proteins, and, most important, lose their ability to multiply. Critically short telomeres therefore function as a tumor suppressor mechanism.<sup>3</sup>

### REGULATION OF TELOMERE LENGTH

Variation in leukocyte telomere length (LTL) is seen interindividually, in different tissues and even on separate chromosomes, and is genetically programmed.<sup>1,2</sup> The attrition rate during life is also thought to be dependent on the initial length of the telomeres. At birth, telomeres are of the same length in boys and girls; later in life, however, they are proportionately longer in women, especially in the premenopausal years compared with men the same age—an effect attributed to estrogen. Also, the TBPs are believed to

exert an effect on the rate of telomere shortening and to prevent premature replicative senescence. It has been debated whether short LTL is a good marker of chronological aging.<sup>4,5</sup>

The more cell divisions that take place in humans (ie, the older persons become), the shorter the telomeres. First, the built-in end replication problem shortens the telomeres, but they are also exposed to reactive oxygen species, which have a predilection for the single-stranded, G-rich telomere overhang.<sup>6</sup> This is an important variable associated with aging and with accelerating the telomere attrition rate. Thus, LTL can be regarded as a marker of biologic aging. Unhealthy lifestyle conditions such as smoking and obesity—both of which are associated with low-grade inflammation, a greater leukocyte proliferation rate, and increased reactive oxygen species production—have frequently (but not always) been associated with shorter telomeres in peripheral blood leukocytes.<sup>7</sup>

### LABORATORY METHODS AVAILABLE

There are some conditions with features of early biologic aging that have been associated with shorter LTL, but also some common chronic disease manifestations show the same findings. Most studies, until recently, have been cross-sectional in design and there is a lack of longitudinal studies with repeated measures of LTL. Different laboratory methods are currently available for the determination of relative LTL (quantitative polymerase chain reaction, q-PCR) or absolute LTL, including the proportion of short telomeres (Southern blot), a more costly and labor-intensive method. Other methods also exist, based on immunofluorescence (fluorescence *in situ* hybridization). This has contributed to some controversy because different studies apply different methods that sometimes do not suit each other. Recently, 1 study compared the 2 methodologies of q-PCR and Southern blot based on blind measurements on the same samples from 50 donors that were performed in 2 independent laboratories on 2 different occasions.<sup>8</sup> Both the q-PCR and Southern blots displayed highly reproducible results, as shown by *r* values more than 0.9 for the correlations between results obtained by either method on both occasions. The interassay coefficient of variation measurement for the q-PCR was 6.5%, whereas that of the Southern blot was 1.7%. The authors concluded that the relation between the results generated by Southern blot vs those generated by q-PCR deviated from linearity.<sup>8</sup> For a number of reasons, Southern blot is considered the gold standard, but this method is also more laborious and expensive. It is for these reasons that q-PCR is

used most often in epidemiologic studies, with a few exceptions.

Some skeptics argue that these measurements (q-PCR and Southern blot) are usually carried out on the same cryopreserved DNA, but never on DNA that was isolated from separate blood samples (taken minutes or hours apart). This would be a real validation of the method, because there could be methodological flaws with the q-PCR method. This comes as no surprise when keeping in mind that the telomere sequence TTAGGG is not amplified by complimentary polymerase chain reaction primers but by rather complex GGTTTTTTGAGGGTGA . . . ' 37-oligomers. Therefore, negative studies can easily occur when the methodology is not clean.

### IS LTL IN PERIPHERAL BLOOD A GOOD MARKER OF TISSUE TELOMERE LENGTH?

Another problem is whether telomere length determination in peripheral blood cells (leukocytes, lymphocytes) really reflects the tissue cell telomere length, for example, in the cardiovascular system. Some publications have stated that such a correlation really exists, for example, between LTL in peripheral blood cells and the vascular tissue.<sup>9</sup> In concordance with this, there is evidence that the synchrony in telomere length between leukocytes and various tissues of healthy humans is remarkably strong.<sup>10</sup>

In 1 study, however, LTL derived from peripheral blood was associated with the presence of atherosclerotic carotid plaques yet was not a proxy for local plaque telomere length. In that study, plaque telomere length was related to plaque characteristics and development of restenosis after endarterectomy in a group of patients undergoing angiography and intervention.<sup>11</sup>

### RISK FACTORS, DISEASE, AND TELOMERE LENGTH

In a number of observational studies, it has been documented that short telomeres or the genetics regulating telomeres are associated with hypertension,<sup>12</sup> type 1 diabetes mellitus,<sup>13</sup> type 2 diabetes mellitus,<sup>14</sup> and CVD manifestations,<sup>15</sup> such as risk of coronary heart disease (CHD),<sup>16</sup> carotid atherosclerosis,<sup>17</sup> stroke,<sup>18</sup> and abdominal aortic aneurysm.<sup>19</sup> This could be the result of the association with intermediate phenotypes, such as obesity, hypertension, or chronic inflammation, or because of the influence of a less healthy lifestyle, including smoking.<sup>7</sup> Previous studies have shown a negative association between telomere length and abdominal obesity and smoking,<sup>20</sup> but also an association with adverse psychosocial factors.<sup>21</sup> In addition, shorter telomere length has been associated with markers of early vascular aging syndrome,<sup>22</sup> such as increased pulse

pressure—a marker of arterial stiffness and a core component of early vascular aging syndrome.<sup>23</sup>

### CONTROVERSIAL RESULTS

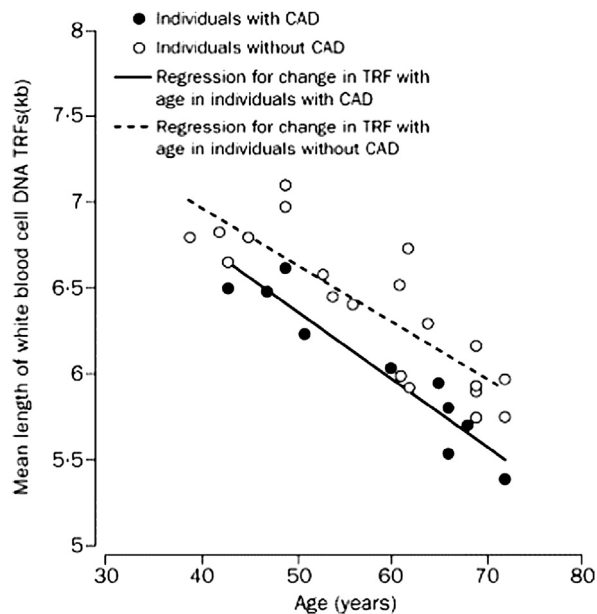
Some findings, however, have also been contradictory or even paradoxical. For example, longer telomeres have been found in disease conditions in which the hypothesis was to find shorter telomeres (because shorter length is often, but not always, associated with increased risk of disease). Therefore, cross-sectional data on measurements of telomere length are obviously not enough to evaluate the full importance of time-dependent changes associated with cardiovascular risk. It thus takes longitudinal studies to compare the telomere attrition rate over time in defined subjects with varying cardiovascular risk. Past studies have shown that 15%–25% of screened subjects might even increase their telomere length over time,<sup>24</sup> possibly as a result of to an increased activity of telomerase or the influence of shifting telomere length in different subpopulations of mononuclear cells. This has been criticized, however, because aging displays a unidirectional progression, and it seems unlikely that LTL elongates with age. LTL elongation in longitudinal studies, therefore, reflects measurement errors of LTL primarily in relation to the duration of follow-up periods.<sup>25</sup>

The influence of cardiovascular drugs on telomere biology and related changes should also be explored further (eg, in relation to treatment by antihypertensive<sup>26</sup> or lipid-lowering drugs<sup>27</sup>). Also, antidiabetes treatment should be tested in a similar way because hyperglycemia is believed to increase tissue aging and the stiffening of arteries.<sup>28</sup>

### TELOMERE LENGTH AND CARDIOVASCULAR RISK

In numerous past studies, shorter LTL has been found in various conditions associated with CVD.<sup>16,29,30</sup> A pilot study found that patients with early-onset coronary artery disease (CAD) with premature myocardial infarction (MI) had a shorter mean LTL compared with healthy subjects of the same age and gender (Fig 1). Furthermore, although both groups experienced telomere attrition with aging, there was a residual difference that served as a marker of increased cardiovascular risk in those patients with early CAD.<sup>31</sup>

In another study of patients with premature MI, a marked reduction in mean LTL was demonstrated compared with healthy control subjects, with a consequent calculated reduction in biologic age of 11.3 years.<sup>32</sup> This was also the result of a study that associated shorter LTL with an increase in prospective risk of MI.<sup>33</sup> The West of Scotland Primary Prevention



**Fig 1.** Plot of mean length of leukocyte DNA terminal restriction fragments vs age of individuals with severe coronary artery disease or normal coronary arteries. CAD, coronary artery disease; TRF, terminal restriction fragments. From reference 31. Reprinted with permission.

Study also demonstrated that LTL is a predictor of future CHD events in middle-age, high-risk men. However, in patients randomized and treated with a statin (pravastatin), the increased risk associated with shorter telomeres was attenuated substantially during follow-up.<sup>27</sup>

It has also been shown that LTL is shorter in patients with carotid plaques compared with those without.<sup>34</sup> However, further studies are needed to compare peripheral measurements (in which rapid cell replication occurs frequently) with those in central tissues (which are less accessible for testing) to determine whether there is a close correlation between these measurements (ie, rate of attrition), as indicated previously.<sup>9</sup> It has even been postulated that there might exist differential aging in different organs, as noticed in kidney transplantation recipients.

#### POSITIVE AND NEGATIVE FINDINGS ON LTL AND CORONARY RISK

A number of newer studies have been published on the association between LTL and ischemic heart disease (IHD). In a large but cross-sectional study, associations between LTL and CHD were evaluated when comparing 598 white male patients (<60 years) who survived a first MI and 653 age-matched control subjects from across Europe.<sup>35</sup> In addition, 413 coronary artery bypass graft patients and 2 groups of 461 patients with familiar hypercholesterolemia (FH), of whom 162

had premature CHD, were recruited. LTL was measured using a real-time q-PCR. It was reported that LTL was significantly shorter in patients (7.85 kb) compared with control subjects (8.04 kb,  $P = 0.04$ ). In the coronary artery bypass graft substudy, LTL was also significantly shorter compared with control subjects. In the FH patients, LTL was shorter in those with CHD (overall, 8.68 kb) compared with the non-CHD subjects (9.23 kb,  $P = 0.012$ ). On the other hand, LTL was not associated with any measured CHD risk factors except for age. These data confirm that subjects with CHD have shorter telomeres than control subjects, and extends this to those with monogenic and polygenic forms of CHD, such as FH.<sup>35</sup>

There are, however, also some negative study findings, which should be acknowledged and form a basis for critical analyses and design of new studies. For example, in 1 Italian study, 199 patients age 18 years–48 years with a first diagnosis of acute MI were enrolled and were matched with 190 control subjects for sex and age.<sup>36</sup> LTL was measured at enrollment using a q-PCR-based method. No significant differences in LTL were observed between cases and control subjects, and with the presence of CAD in patients. Furthermore, multivariate regression analysis showed LTL to be significantly shorter in hypertensive patients than in normotensive subjects ( $P = 0.04$ ). In all, 171 patients completed the average follow-up of 9 years, during which time 92 of them presented with a major cardiovascular event. During multivariate regression analysis, the LTL determined at enrollment did not predict major cardiovascular events. The authors concluded that LTL did not represent a marker of acute MI in young patients and was not predictive during follow-up.<sup>36</sup> This could, however, have been influenced by the relatively young age and low number of study participants as well as other methodological shortcomings. For example, no correlation with chronological age and LTL was shown, as would have been expected.

To settle this controversial issue, large-scale, population-based cohorts are needed. In Denmark, this was achieved in 19,838 subjects from the general population belonging to the Copenhagen City Heart Study and the Copenhagen General Population Study.<sup>37</sup> Participants were monitored for up to 19 years for incident MI ( $n = 929$ ), IHD ( $n = 2038$ ), and death ( $n = 4342$ ), with a complete follow-up of all subjects. Results indicated that LTL decreased linearly with increasing age in women and men in both studies, as expected. Multifactorial adjusted and significant hazard ratios (HRs) were 1.10 for MI, 1.06 for IHD, and 1.09 for early death per 1000-bp decrease in telomere length. The multifactorial adjusted HRs for the shortest vs the longest decile of telomere length were also significant, along with the

HRs for MI (1.49), for IHD (1.24), and for early death (1.25). The authors concluded that short telomere length is associated with only modestly increased but independent risk of MI, IHD, and early death.<sup>37</sup> Because this is a very large study, and one of the best to date, the results seem to be robust and trustworthy.

One prospective study has indicated a relationship between telomere biology and risk of CHD in patients with acute coronary syndrome.<sup>38</sup> In addition, telomerase activity in leukocytes has been associated with the presence of calcified atherosclerotic coronary plaque, and was also a predictor of progression of plaque among persons with short telomeres.<sup>39</sup> Also, shorter LTL is associated with high-risk plaque morphology on virtual histology intravascular ultrasound, but not total 3-vessel plaque burden in CAD.<sup>39</sup> Monocytes with disrupted telomeres show increased proinflammatory activity, which is also seen in CAD patients, suggesting that telomere shortening promotes or is associated with high-risk plaque subtypes by increasing proinflammatory activity.<sup>40</sup>

It has been suggested that situational changes in cellular subpopulations could have a significant impact on mean telomere length without actually affecting LTL in the cell populations themselves. In the acute phase of MI, for example, patients develop monocytosis and granulocytosis, paralleled by temporary lymphopenia. Therefore, measurements of mean LTL in these patients could automatically render wrong results when comparing them with healthy control subjects or stable patients with CHD. This should be explored further in future studies.

#### PARADOXICAL FINDINGS IN RELATION TO LEFT VENTRICULAR HYPERTROPHY

As previously indicated, there are some paradoxical findings that are not easily explained. For example, LTL increases in some subjects during follow-up, and it is unclear why this occurs; however, this is most likely to be an artifact. In addition, 2 population-based studies have shown that subjects with left ventricular hypertrophy (LVH) have longer telomeres compared with control subjects, 1 in the United States<sup>41</sup> and 1 in Belgium.<sup>42</sup> This is counterintuitive because LVH is usually a marker of target organ damage and underlying risk factor burden, most notably longstanding hypertension in poor control. One possible explanation could be that cell hypertrophy associated with LVH is not associated with frequent cell divisions, as found in tissues with hyperplasia. In addition, data on the relationship between hypertension and telomere length are inconsistent, with some studies showing an inverse association between elevated blood pressure or hypertension and shorter LTL and other studies that do not. This could potentially reflect different origins of the elevated

blood pressure, or the age range of study participants (eg, whether hypertension is associated with obesity and insulin resistance).

#### A POPULATION-BASED STUDY IN SWEDEN ON MYOCARDIAL FUNCTION

In a population-based study from Malmö, Sweden, we compared relative TL (RTL) with left ventricular mass and ejection fraction (EF). In addition, the RTL in relation to other markers of cardiovascular risk was investigated, including blood pressure and glucose. The study subjects belonged to a large cohort ( $n = 1792$ , 70% males) of middle-age to elderly subjects, examined by echocardiography. These were mostly elderly subjects free of previous MI, stratified for increasing levels of hyperglycemia and/or type 2 diabetes mellitus. Methods as well as various definitions used have been described previously by Leosdottir et al.<sup>43</sup> The echocardiographic examination was carried out by use of tissue Doppler imaging. The left ventricular mass index (LVMI) and EF were calculated, as described in detail.<sup>43</sup> The method used for assessment of RTL was q-PCR, described previously by Cawthon.<sup>44</sup>

#### RESULTS ON LTL IN RELATION TO LVM AND BLOOD PRESSURE

All subjects ( $n = 204$ ) that were T/S, where T is the amount of telomere and S is the amount of a single copy gene in each well outliers (outside T/S range, 0.5–2.5) or had unrealistic data on blood pressure levels (based on extreme values) or LVMI were excluded. Thus, a total of 1588 subjects remained, including 570 subjects (35.9 %) with type 2 diabetes mellitus. The main clinical characteristics of the study sample are presented in Table I. RTL was associated inversely with age ( $P = 2.37 \times 10^{-30}$ ), adjusted for gender. RTL was also associated negatively with smoking status ( $P = 0.049$ ) and fasting plasma glucose ( $P = 0.032$ ) in all subjects. In females, RTL tended to be longer than in males, but this association was of borderline significance after adjusting for age and smoking ( $P = 0.054$ ). RTL, adjusted for age and gender, was associated positively with levels of systolic and diastolic blood pressure ( $P = 0.045$  and  $P = 0.011$ , respectively). These correlations were still significant after adjusting for additional confounders, such as body mass index, smoking and use of antihypertensive treatment (Table II). In addition, RTL was longer in hypertensive subjects compared with normotensive control subjects ( $P = 0.038$ ; Table III). Furthermore, RTL was neither correlated to LVMI ( $P = 0.9$ ) nor to EF ( $P = 0.8$ ) or pulse pressure ( $P = 0.3$ ) after adjusting for age and gender in the whole cohort.

We concluded, therefore, that LTL in these elderly subjects (representing survivors aged 70 years–75 years)

**Table 1.** Clinical characteristics of the Malmö Preventive Project subjects (n = 1588)

Characteristic			Nondiabetic subjects		Subjects with diabetes mellitus	
	Men	Women	Men	Women	Men	Women
n	1110	478	674	344	436	134
Age, years; mean (SD)	66.9 (6.0)	69.5 (5.1)	66.5 (6.1)	69.1 (5.2)	67.7 (5.8)	70.5 (4.7)
BMI, kg/m <sup>2</sup> ; mean (SD)	28.2 (4.0)	28.5 (5.1)	27.5 (3.6)	27.8 (4.8)	29.5 (4.2)	30.4 (5.4)
SBP, mmHg; mean (SD)	148.1 (19.7)	144.8 (20.0)	147.3 (19.1)	143.5 (19.2)	149.5 (20.5)	148.1 (21.7)
DBP, mmHg; mean (SD)	85.1 (10.3)	82.5 (10.1)	85.6 (10.2)	82.6 (10.2)	84.3 (10.4)	82.2 (9.9)
fP-glucose, mmol/L; mean (SD)	7.0 (2.1)	6.4 (1.9)	5.9 (0.6)	5.7 (1.0)	8.6 (2.5)	8.3 (2.3)
LDL-C, mmol/L; mean (SD)	3.38 (0.99)	3.65 (1.00)	3.55 (0.95)	3.79 (0.97)	3.13 (1.00)	3.29 (0.99)
HDL-C, mmol/L; mean (SD)	1.23 (0.34)	1.53 (0.47)	1.29 (0.34)	1.58 (0.47)	1.14 (0.32)	1.42 (0.45)
EF, %; mean (SD)	59.1 (8.1)	62.3 (6.6)	59.3 (7.7)	62.5 (6.9)	58.7 (8.7)	61.7 (5.9)
LVMI, g/m <sup>2</sup> ; mean (SD)	95.3 (25.3)	83.6 (20.5)	94.2 (24.7)	82.0 (19.4)	97.1 (26.1)	87.7 (22.6)
Relative telomere length, T/S; mean (SD)	1.16 (0.34)	1.16 (0.34)	1.17 (0.34)	1.18 (0.35)	1.14 (0.34)	1.10 (0.31)
Hypertension at examination, %	80.6	79.9	75.8	77.0	88.1	87.3
Current smoker, %	47.7	31.4	48.4	31.8	46.6	30.5
On antihypertensive treatment, %	49.6	50.0	40.8	46.2	63.3	59.7

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; EF, ejection fraction; fP-glucose, fasting plasma glucose; HDL-C, high-density cholesterol; LDL-C, low-density cholesterol; LVMI, left ventricular mass index; SBP, systolic blood pressure; SD, standard deviation; T/S, amount of telomere/amount of a single copy gene in each well.

Hypertension is defined as systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg or use of antihypertensive treatment.

was not associated with left ventricular mass, but was associated positively with hypertension and blood pressure levels in elderly subjects with normal glucose tolerance, not seen in subjects with type 2 diabetes mellitus. Our data thus suggest that subjects with hypertension have longer telomeres than normotensive subjects, at least in the elderly. No associations were observed between RTL and LVMI or pulse pressure, both variables being influenced by elevated blood pressure level. In subjects with established type 2 diabetes mellitus, no association was found between RTL and blood pressure levels, gender, and smoking. These results might indicate that telomere length is a stronger indicator of cardiovascular risk factors in subjects with normal glucose tolerance. In subjects with diabetes mellitus, however, the association between RTL and the different variables analyzed may be obscured by the influence of poor glucometabolic control. In the whole cohort, RTL showed an inverse relation to fasting plasma glucose. However, this relation was absent in the nondiabetic group. When comparing subjects with and without type 2 diabetes mellitus, RTL was longer in the nondiabetic group, although this association did not reach significance after full adjustments.

Regardless of study limitations, RTL was still strongly correlated to age in the whole sample based on multiple regression analysis. There was a highly significant inverse relationship between RTL and age ( $P = 2.37 \times 10^{-30}$ ), after adjusting for gender, despite a rather high variation coefficient (17%). Because this is a well-known and expected correlation used as a meth-

odological control in earlier telomere studies, we believe our data to be accurate. Another advantage of the current study is the size of the study population, which is unusually large, with 1588 subjects undergoing tissue Doppler imaging echocardiography. LVM and pulse pressure are often regarded as markers of long-standing hypertension. Further studies, preferably based on repeated measures of telomere length over time, are needed to expand our knowledge concerning the biology of telomeres and its associations with cardiovascular risk factors and disease as well as associations with cardiac morphology and function across the life span. There might even exist an interaction with genetic markers (ie, the angiotensin converting enzyme insertion/deletion polymorphism) in influencing telomere length and LVH,<sup>45</sup> and this may be of special relevance in essential hypertension.<sup>46</sup>

We can thus conclude that RTL was related inversely to age but not with measures of cardiac morphology or function (LVMI, EF) in a cross-sectional, observational study in elderly subjects from an urban population, stratified for increasing levels of fasting plasma glucose and diabetes mellitus. Furthermore, RTL was associated positively with blood pressure levels and hypertension status in subjects with normal glucose tolerance, but not in subjects with diabetes mellitus. In the whole cohort, RTL was associated inversely with fasting plasma glucose levels. Future studies should investigate these associations preferably based on repeated measures and changes of RTL (attrition rate) over time.

**Table II.** Cross-sectional associations between relative telomere length and cardiovascular risk factors including LVMI

Variable	Model covariates for adjustment	All subjects		Nondiabetic participants		Participants with diabetes mellitus	
		$\beta$	P value	$\beta$	P value	$\beta$	P value
Age, year	Sex	-0.29	< <b>0.001</b>	-0.303	< <b>0.001</b>	-0.255	< <b>0.001</b>
BMI, kg/m <sup>2</sup>	Age, sex	-0.019	0.43	-0.008	0.78	-0.012	0.77
	Age, sex + smoking + AHT	-0.029	0.23	-0.022	0.47	-0.016	0.70
SBP, mmHg	Age, sex	0.049	<b>0.045</b>	0.055	0.07	0.047	0.24
	Age, sex + BMI + smoking + AHT	0.049	<b>0.044</b>	0.057	0.06	0.043	0.30
DBP, mmHg	Age, sex	0.062	<b>0.01</b>	0.068	<b>0.026</b>	0.052	0.21
	Age, sex + BMI + smoking + AHT	0.068	<b>0.007</b>	0.073	<b>0.018</b>	0.052	0.22
PP, mmHg	Age, sex	0.024	0.33	0.028	0.35	0.031	0.45
	Age, sex + BMI + smoking + AHT	0.022	0.38	0.028	0.36	0.025	0.55
fP-glucose, mmol/L	Age, sex	-0.054	<b>0.027</b>	-0.006	0.84	-0.074	0.07
	Age, sex + BMI + smoking + AHT	-0.054	<b>0.032</b>	-0.001	0.98	-0.073	0.08
LDL-C, mmol/L	Age, sex	0.038	0.13	0.023	0.45	0.047	0.26
	Age, sex + BMI + smoking + AHT	0.044	0.09	0.034	0.29	0.043	0.33
HDL-C, mmol/L	Age, sex	0.006	0.80	0.006	0.84	-0.016	0.70
	Age, sex + BMI + smoking + AHT	0.005	0.87	0.008	0.80	-0.019	0.68
LVMI, g/m <sup>2</sup>	Age, sex	0.002	0.94	0.025	0.44	-0.027	0.53
	Age, sex + BMI + smoking + AHT	0.005	0.85	0.019	0.56	-0.013	0.77
EF (%)	Age, sex	0.005	0.84	-0.024	0.45	0.047	0.27
	Age, sex + BMI + smoking + AHT	0.002	0.93	-0.022	0.49	0.042	0.33

Abbreviations: AHT, antihypertensive treatment; BMI, body mass index; DBP, diastolic blood pressure; EF, ejection fraction; fP-glucose, fasting plasma glucose; HDL-C, high-density cholesterol; LDL-C, low-density cholesterol; LVMI, left ventricular mass index; PP, pulse pressure; SBP, systolic blood pressure.

Multivariate linear regression analysis with significant correlations are indicated in bold type.

### NEED FOR LONGITUDINAL STUDIES ON TELOMERE ATTRITION

As stated previously, most existing studies have been cross-sectional in nature, and further follow-up studies with repeated measurements of LTL are needed to understand more completely the interplay between cardiovascular risk and changes in the biology and length of telomeres (Fig 2). Just a few longitudinal studies with repeated measurements of telomere length are currently available. In 1 such population-based study from Malmö, Sweden, it was shown that changes in relative LTL (telomere attrition) over 15 years was associated with markers of cardiovascular aging, such as increased risk of MI, treatment with antihypertensive drugs (as a marker of longstanding hypertension), and elevated brachial pulse pressure, based on preliminary data presented at the European Society of Hypertension XXI annual meeting in Milan, June 2011.<sup>47</sup>

### GENETICS AND FAMILY TRAITS OF TELOMERE LENGTH

Another way to try to disentangle the causality problem that relates to telomere biology and CVD is to look for the genetic factors regulating telomere biology as well as family traits of telomere length in relation to disease. Previous genomewide association studies identified 2 loci on chromosomes 3q26.2 (telomerase RNA component [*TERC*]) and 10q24.33 (oligonucleotide/oligosaccharide-binding folds containing one gene [*OBFC1*]) that are associated with the inter-individual LTL variation. In a meta-analysis of 9190 individuals from 6 independent genomewide association studies and validation in 2226 individuals from 4 additional studies, the genetic architecture of inter-individual LTL variation in the general population was described, showing the genetic influence and thus hereditary traits.<sup>2</sup> In another cohort study prospective CVD mortality was investigated in relation to single nucleotide polymorphisms in *OBFC1* and *TERC*,

**Table III.** Multiple logistic regression analysis for relative telomere length correlations with categories of gender, hypertension, type 2 diabetes mellitus, and smoking

Categories	Model covariates	All subjects		Nondiabetic participants		Participants with diabetes mellitus	
		$\beta$	P value	$\beta$	P value	$\beta$	P value
Gender	Age	0.493	<b>0.013</b>	0.638	<b>0.009</b>	0.075	0.83
	Age + smoking	0.39	<b>0.054</b>	0.56	<b>0.024</b>	-0.102	0.78
Hypertension	Age, gender	0.471	<b>0.038</b>	0.704	<b>0.008</b>	0.007	0.99
	Age, gender + smoking + BMI + AHT	0.567	<b>0.034</b>	0.757	<b>0.016</b>	0.084	0.87
DM2	Age, gender	-0.259	0.17	NA	NA	NA	NA
	Age, gender + smoking + hypertension + BMI + AHT	-0.317	0.11				
Current smoker	Age, gender	-0.361	<b>0.049</b>	-0.414	0.072	-0.277	0.36
	Age, gender + hypertension + AHT	-0.37	<b>0.044</b>	-0.453	<b>0.05</b>	-0.275	0.37

Abbreviations: AHT, antihypertensive treatment; BMI, body mass index; DM2, type 2 diabetes mellitus; NA, not applicable.

Hypertension is defined as systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg or presence of antihypertensive treatment. Diabetes mellitus is defined as a history of diabetes mellitus by questionnaire or use of antidiabetic treatment or fasting plasma glucose  $\geq 7.0$  mmol/L at 2 separate measurements or fasting plasma glucose  $\geq 11.1$  mmol/L at 1 measurement.

All significant correlations are indicated in bold.

genes related to LTL, among 3271 white participants age 65 years or older.<sup>48</sup> The findings supported the hypothesis that telomere biology and associated genes may play a role in CVD-related death, particularly among women. In addition, it has been shown that genes encoding *TERC* are associated with both LTL and longevity in humans.<sup>49</sup>

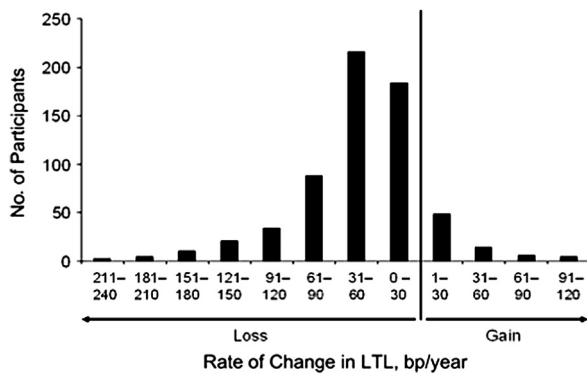
Telomerase activity is exhibited in gametes and stem and tumor cells. In human somatic cells, proliferation potential is strictly limited and senescence follows approximately 50 cell divisions–70 cell divisions. Telomerase activity is regulated in a complex way during the life course, as reviewed.<sup>50</sup> Hills and Lansdorp<sup>51</sup> performed a detailed study of the telomere length by flow fluorescence *in situ* hybridization analysis in leukocytes from 835 healthy individuals and 60 individuals with reduced telomerase activity. Healthy individuals showed a broad range in average telomere length in granulocytes and lymphocytes at any given age. The average telomere length declined with age at a rate that differed between age-specific breakpoints and between cell types. Heterozygous carriers for mutations in either the telomerase reverse transcriptase or the *TERC* gene displayed striking and comparable telomere length deficits. Furthermore, noncarrier relatives of such heterozygous individuals had somewhat shorter leukocyte telomere lengths than expected; this difference was most profound for granulocytes.<sup>51</sup> Failure to maintain telomere homeostasis as a result of partial telomerase deficiency is thought to trigger cell senescence or cell death, eventually causing tissue failure syndromes.

Family studies have not been unequivocal, and different findings have been presented. In the Belgian Asklepios study, LTL was measured using telomere restriction fragment analysis (Southern blot) in the young to middle-aged (~35 years–55 years), free from overt CVD and from the general population, and could be combined with data on family history of CVD. No shorter LTL could be found in healthy subjects with a positive family history of CVD compared with those without, and the authors concluded that these findings cast serious doubt on the hypothesis that LTL is shorter in families with an increased risk of CVD and do not, in their opinion, support the telomere hypothesis of CVD.<sup>52</sup> However, in patients with ischemic heart failure, reduced LTL was recorded in their offspring, suggesting a potential causal link of LTL in IHD. In addition, strong correlations were found in another study between parent and offspring LTL in all examined cell types, suggesting high heritability of LTL among cell types.<sup>53</sup>

## CONCLUSIONS

Research in telomere biology may help elucidate ways to measure cardiovascular aging, based not only on physiological changes but also by use of biomarkers,<sup>54</sup> in which determination of LTL could be of some interest. There is no doubt that aging itself is of major importance in understanding how the vascular tree and the myocardium change at a cellular level in all humans. This has been described in a recent review on aging and atherosclerosis, noting mechanisms, functional consequences, and potential therapeutics for cellular





**Fig 2.** Distribution of the rate of change in leukocyte telomere length (LTL) in Louisiana study participants. A total of 561 participants showed LTL shortening, and 74 showed either gain ( $n = 68$ ) or no change ( $n = 6$ ) in LTL between baseline (1995–1996) and follow-up (2001–2006) examinations. From reference 24. Reprinted with permission.

senescence.<sup>55</sup> It is, however, not sufficient to measure LTL in cross-sectional studies only and to analyze correlations or associations. Instead, there is a need to do repeated measurements during follow-up studies or in relation to different drug treatments. Furthermore, other aspects of telomere biology should also be measured (eg, telomerase) because LTL is longer in patients with a better physical capacity, indicating a change in telomerase activity related to lifestyle.<sup>56</sup> This has also been shown as a result of improved lifestyle for 3 months in a pilot study in 30 men with prostatic cancer when increased telomerase activity was recorded.<sup>57</sup>

The genes regulating telomere biology should be tested for associations with CVD manifestations to prove causality between the biomarker (telomere length), and the disease manifestation, which would be possible if Mendelian randomization principles are used.<sup>58</sup>

In cardiovascular medicine, the concept of the cardiovascular aging continuum has now emerged as useful.<sup>59</sup> This is also why research needs to be developed to find out more about the telomere system in health and disease, especially in relation to CVD<sup>16</sup> and other chronic disease conditions.<sup>60</sup>

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