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Telomere shortening & metabolic/vascular diseases

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Telomeres are specialized DNA-protein structures located at the ends of eukaryotic chromosomes whose length is progressively reduced in most somatic cells during ageing. Over the past decade, emerging evidence has shown that the telomeres are essential regulators of cellular life span and chromosome integrity in a dynamic fashion. By inducing genomic instability, replicative senescence and apoptosis, shortening of telomeres is thought to contribute to organismal ageing. While the aetiology of cardiovascular diseases and diabetes represent a complex interaction between various risk factors overlaid on different genetic backgrounds, the conventional risk factors often did not explain the inter-individual variability related to predisposition of disease states. This underscores the need for biological indicators of ageing in evaluating the aetiology of several age-related disorders, and recent studies indicate that telomere length could qualify as an ideal marker of biological ageing. Short telomeres have been detected in senescent endothelial cells and vascular smooth muscle cells from human atherosclerotic plaque as well as in myocardial tissue from patients with end-stage heart failure and cardiac hypertrophy. In addition, telomere shortening has been demonstrated in WBCs from patients with coronary heart disease, premature myocardial infarction, hypertension and diabetes mellitus. In this review, we discuss the telomere hypothesis of ageing as well as human studies that address the role of telomeres in cardiovascular, diabetes and other cardio-metabolic pathologies.

Key words Atherosclerosis - CVD - diabetes - oxidative stress - telomere shortening

Telomeres are snippets of DNA at the ends of chromosomes that function in part like the plastic tips on the ends of shoelaces, preventing chromosomal fusions and offering genomic integrity and stability. Apart from ensuring chromosome stability, telomeres also provide a mechanism for 'counting' cell divisions, and thus signal replicative senescence.

The telomeric complex is composed of (*i*) non-coding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in humans) that are extended several thousand base pairs and end in a 3'end single-stranded overhang, (*ii*) the enzyme telomerase, and (*iii*) several telomere repeat binding factors (TRF1, TRF2, *etc*.) with structural, and regulatory roles that participate in the control of

telomere length and capping[1]. Since conventional DNA polymerases responsible for the majority of DNA replication in eukaryotic cells, are unable to synthesize the last stretch of DNA on the lagging strand, it was proposed that chromosomal ends progressively shorten with each replication cycle – a phenomenon known as the "end replication problem". Hence the terminal replication of chromosomes requires a specialized polymerase termed telomerase. Telomerase has two components, a catalytic telomerase reverse transcriptase (TERT) and a telomerase RNA component (TERC) that serves as a template for the synthesis of new telomeric DNA repeats[2].

Telomere protection depends upon several factors such as its precise protein composition, telomere length, and telomerase activity level. The probability of telomere uncapping increases when one or more of these parameters are critically altered and cannot be compensated by the others. Telomerase expression and activity and telomere length are regulated in a tissue-specific and developmental manner in several species, including humans[1]. In general, these parameters are greater during embryonic development and become low or undetectable after birth, although significant differences in adult tissues have been reported. Telomerase activity is also regulated at different molecular levels, including transcription, mRNA splicing, maturation and modification of TERT and TERC and epigenetic pathways[3]. Progressive telomere shortening in cell culture and during ageing of the whole organism is a characteristic of most adult somatic cells, which exhibit low or no telomerase activity. In contrast to adult somatic cells, the extended proliferative capacity of germ and tumour cells correlates with maintenance of high telomerase activity and long telomeres[2]. Thus telomeres have been proposed to serve as a molecular device that counts the number of cellular divisions and limits life span[4].

Telomeres and ageing

Recent research points to the crucial roles of telomeres and telomerase in cellular ageing and potentially in disease. Ageing is a process associated with progressive changes, ultimately leading to death, and the mechanisms involved in ageing are still far from well understood. The search for ageing and longevity genes has long been a focus in biomedical research. Since telomeres shorten as a function of age *in vivo* and telomerase antagonizes the process of telomere shortening, whether or not telomere-shortening serves, as a timer with different settings in different species to control the onset of cell senescence, and thus life span, has been the subject of intense debate[5].

A well established model for the analysis of ageing at the cellular level is *in vitro* cultivation of human diploid cells that divide a limited number of times before undergoing a state called "cellular senescence". This limit has been named "the Hayflick limit". Such cells are irreversibly blocked in G1 phase of the cell cycle and become unresponsive to mitogenic stimuli yet can remain viable and metabolically active. The senescent phenotype is accompanied by dramatic changes in morphology, nuclear structure, gene expression, protein processing and metabolism. An increased fraction of these cells positively stained for senescence-associated β-galactosidase and tumour suppressors such as p53, p21 and p16 is upregulated. This cellular senescence thus, represents a tumour suppressor mechanism[6]. Early studies in mice bearing a germ line knockout of the mTR gene and thus null telomerase activity show that short telomeres trigger multiple ageing related processes including cell growth arrest, apoptosis and decreased capacity in response to stresses in highly proliferative organs, demonstrating a critical role for telomere length in genomic stability, cell replicative life span and ageing[5]. The consequences of telomere ablation at the organismal level have been rigorously assessed in *TERC*-deficient mice[7] which undergo progressive telomere shortening with each generation and lose viability when they reach critically short telomeres (typically after 3 to 5 generations). Remarkably, late generation *TERC*-null mice display premature ageing symptoms and associated disorders, such as infertility, hair graying, alopecia, heart dysfunction,

hypertension, various tissue atrophies and decreased tissue regeneration capacity. These findings indicate that a minimal telomere length is required to maintain tissue homeostasis in the mouse and lend support to the notion that progressive telomere shortening may be involved in the pathogenesis of age-related human disorders.

Telomeres and metabolic/vascular diseases

Recent studies indicate that telomere biology is intimately linked to the genetic/environmental aetiology of cardiovascular and metabolic diseases and telomere shortening is emerging as an important biomarker at the interface of cardiometabolic diseases (Table I).

Telomere shortening in cardiovascular diseases (CVDs): Today, there is increasing evidence of an association between telomere length and many disease states. Coronary heart disease (CHD) is a chronic disease in which the coronary arteries become 'hardened' and the lumen narrowed by the development and progression of atherosclerosis.

Table I. Human studies showing associations between telomere shortening and vascular/metabolic diseases

Cell type	Main findings	Year	Ref
EC	Telomere loss in human vascular disease	1995	8
Leukocytes	Telomere shortening in respiratory chain disorders	1997	9
Leukocytes	Telomere shortening in type 1 diabetic patients	1998	24
Leukocytes	Short telomeres in vascular dementia	2000	12
Leukocytes	Biology of cardiovascular aging differs between men and women	2001	19
Leukocytes	Telomere shortening in severe CAD	2001	13
HAEC	Loss of telomere induces endothelial dysfunction	2002	11
PBMC	Telomere shortening in CAD with metabolic disorders	2003	16
Leukocytes	Short telomeres with risk of premature MI	2003	17
Coronary EC	Telomere shortening in CAD	2004	10
Leukocytes	Telomere shortening in CAD with plaques	2004	14
Leukocytes	Telomere shortening correlating with cardiovascular damage	2004	27
PBMC	Telomere shortening in response to life stress	2004	18
Leukocytes	Telomere shortening in type 2 diabetic patients	2005	25
		2007	21
Leukocytes	Menopause impacts telomere length and its relation to IR and inflammation	2006	30
Leukocytes	Rise in BMI causes telomere attrition	2005	29
Leukocytes	Obesity and smoking accelerates human ageing	2005	31
Leukocytes	Aldosterone might be linked to telomere attrition and perhaps increased biological ageing	2005	28
Leukocytes	Telomere shortening in calicific aortic valve stenosis	2006	15
Leukocytes	Telomere shortening in IR & hypertension	2006	20
Monocytes	Telomere shortening in type 2 diabetes	2006	26
Leukocytes	Telomere shortening in Cardiovascular Health Study (CHS)	2006	22
Leukocytes	Telomere shortening in IGT subjects	2007	21
Leukocytes	Type 2 diabetic subjects with carotid plaques exhibit shorter telomeres	2007	21
Leukocytes	Telomere length is associated with future CHD	2007	23

EC, endothelial cell; HAEC, human aortic endothelial cell; PBMC, peripheral blood mononuclear cell; MI, myocardial infarction; CAD, coronary artery disease; IR, insulin resistance; IGT, impaired glucose tolerance

Develolpment of CHD is dependent on a number of factors, such as the conventional risk factors (hypertension, smoking, dyslipidaemia, age, positive family history), emerging risk factors [C-reactive protein (CRP), homocysteine, *etc*.] and the effect of the genetic background of the individual. However, despite advances in our understanding of these factors that predispose to CHD, there are many key aspects that remain unclear. These include variation in susceptibility, and a highly variable age of onset in individuals who display very similar risk profiles.

Association of ageing and cardiovascular disease has been a matter of great interest in the field of cardiovascular and geriatric medicine. Atherosclerosis is a common underlying condition, and ageing is considered a major risk factor of atherosclerosis. In addition, ageing related endothelial dysfunction appears to be an important factor that links ageing to cardiovascular diseases. Thus, endothelial dysfunction triggered by atherogenic stimuli (*e.g.*, elevated plasma cholesterol level, hypertension, diabetes, and smoking) is of central importance in the pathogenesis of atherosclerosis. Atherosclerosis is initiated by repeated mechanical, hemodynamic, and/or immunological injury, probably involving oxidative stress, to the mural and focal regions of the endothelium (response-to-injury hypothesis). Such insults may cause augmented cell turnover in certain cell populations or tissues rendering the cells older and nearer to their maximum replicative capacity. It has been shown that human vascular endothelial cells lose telomeres as a function of replicative age and that the telomere loss is greater in tissue susceptible to atherogenesis[8] suggesting that focally enhanced cellular turnover may cause early cellular senescence associated with telomere shortening. Thus, there are compelling data implicating that cellular senescence plays a role in the pathogenesis of atherosclerosis. *In vivo*, age-dependent telomere shortening has been reported in endothelial cells (ECs) from iliac, thoracic, and coronary arteries[8,10,32]. Minamino *et al*[11] reported that vascular ECs with senescence-associated phenotypes are present in human atherosclerotic lesions. Notably, Ogami *et al*[10] found shorter telomeres in coronary ECs of patients with coronary artery disease (CAD) than in age-matched non CAD patients. Collectively, these studies suggest that EC dysfunction and replicative senescence induced by telomere shortening play a critical role in coronary atherogenesis.

Several studies have established an association between telomere length in WBCs and atherosclerosis. Patients with vascular dementia, a disorder that is frequently associated with cerebrovascular atherosclerosis and stroke, exhibit significantly shorter WBC telomeres compared with age-matched controls[12]. Likewise, average telomere length in leukocytes of patients with severe CAD was significantly shorter compared with normal coronary angiograms[13]. It was also shown that hypertensives with carotid artery plaques had shorter telomeres compared to patients without plaques[14]. However, Kurz *et al*[15] have reported that calcific aortic valve stenosis, but not CAD, is associated with shorter leukocyte telomeres in a cohort of elderly patients. A Japanese study also demonstrated that telomere shortening could be involved in the development of atherosclerotic disease in patients with hypercholesterolaemia and diabetes[16]. In a large case-control study, short telomeres increased the risk of premature myocardial infarction by approximately 3-fold[17]. Similarly, there also exists a relationship between telomere length and human hypertension[19,20,33]. More recently, we have shown that type 2 diabetic subjects with atherosclerotic plaques had significantly shorter telomeres compared to diabetic subjects without atherosclerotic plaques[21]. Notably, the cardiovascular health study (CHS) has reestablished the associations between telomere length in leukocytes and indices of sub-clinical and clinical cardiovascular disease[22]. Collectively these studies raise the possibility that telomere attrition may be a primary abnormality that renders the organism more susceptible to cardiovascular risk factors and thus establishes a link between telomere

shortening in WBCs and cardiovascular disease. This indeed, is supported by a recent prospective randomized study (the West of Scotland Primary Prevention Study, WOSCOPS) where it was shown that leukocyte telomere length is associated with future coronary heart disease events in middle-aged, high-risk men[23].

Telomere shortening and diabetes mellitus: Diabetes patients are at higher risk for microvascular and macrovascular disease. Jeanclos *et al*[24] reported that telomere length in WBCs from patients with type 1 diabetes is reduced compared with age-matched non-diabetic control subjects. Adaikalakoteswari and colleagues[25] demonstrated an association of telomere shortening in patients with type 2 diabetes. A recent study lends support to our observations in that monocyte telomere length was significantly shorter in type 2 diabetics compared to control subjects[26]. While our studies demonstrated an association of telomere shortening with systemic markers of oxidative stress (lipid peroxidation and protein oxidation) in type 2 diabetes, the study by Sampson *et al*[26] also showed an association of telomere shortening and oxidative DNA damage. However, unlike our study, the telomere shortening observed by the European group[26] was independent of glycaemic control, insulin resistance (IR) and inflammatory markers. The strong association of telomere shortening and IR shown in our study could imply a role of ethnicity as Indians have been shown to be more insulin resistant compared to their European counterparts[34].

Very recently, we have demonstrated an association between shortened telomeres and impaired glucose tolerance[21]. An increased predisposition to diabetes and coronary artery disease (CAD) among Asian Indians has long been recognized and claimed to stem in most part from IGT[35]. The higher rates of CAD and type 2 diabetes among Indians are also often not explained by traditional risk factors. Therefore, telomere shortening may represent a non-traditional risk factor and long-term biomarker to be associated with IGT and in the natural history of diabetes and cardiovascular diseases. Referring to the recent literature, it appears that telomere attrition is strongly correlated with insulin resistance (IR)[29,30]. Inverse correlations of WBC telomere length with insulin resistance, serum leptin and BMI were also reported in a large-population based cross-sectional study[31]. Since both insulin resistance and obesity prime the genesis of type 2 diabetes and/or cardiovascular disease, it appears that telomere shortening could represent a continuously monitorable biomarker. Moreover, it was inferred from the Framingham Heart Study that shorter leukocyte telomere length in hypertensives is largely due to insulin resistance[20]. There is increasing evidence that IR, a predecessor for both CAD and diabetes, is associated with chronic low grade inflammation and oxidative stress[36]. There might be a continuous genesis of oxidative stress and inflammation in the natural history of diabetes with its perturbations starting as early as IGT[37,38]. Therefore, it is plausible to suggest that these factors could mechanistically connect insulin resistance and impaired glucose tolerance with changes in telomere length.

Telomeres shortening, women and role of estrogen

Several studies confirm that the age-adjusted telomere length is shorter in men than in women[19,21,25,33,39]. As pre-menopausal women are less prone than man to cardiovascular diseases[40] and several systemic parameters show poor correlation with blood pressure in women[41], these studies indicate that the biology of ageing differs between men and women. The lower incidence of cardiovascular disease in pre-menopausal women compared with men may be attributable, at least in part, to estrogens[42]. In addition to the well characterized actions on lipoprotein metabolism and on vascular cells, the influence of estrogen on telomere homeostasis may also contribute to their beneficial effects on the cardiovascular system. This could explain the results of both human and animal studies that revealed higher telomerase

activity and diminished rate of age-related telomere attrition, and thereby longer telomeres in females than in males. Very interestingly the gender difference in telomere length commonly seen, was absent in patients with type 2 diabetes[21,25]. This observation supports the well known fact that women with type 2 diabetes lose their protection from associated diseases[43]. We suggest that the enigmatic gender difference in telomere shortening and the natural history of diabetes need to be explored by longitudinal studies involving both pre- and post-menopausal women. Such studies are important because (*i*) estrogen is a potent anti-inflammatory and antioxidant agent[44], (*ii*) there is an estrogen-response element present in hTERT[45], and (*iii*) hormonal changes in women are expected to have drastic influences on insulin resistance, adiposity, oxidative stress and telomere length.

Telomeres and oxidative damage

Although the telomere length may reflect the history of tissue replication, it is also suggested that mechanisms other than cellular turnover may take part in the regulation of telomere length. Accumulation of oxidative damage is thought to play an important role in aging and age-associated diseases and oxidative stress may function as a common trigger for activation of the senescence programme. Studies report that telomeric DNA sequences are particularly prone to chromosomal breakage and their GGG-triplets are a favourable target for reactive oxygen species (ROS)[46]. Direct administration of oxidants to cells damages DNA, breaks polyguanosine sequences in telomere repeats, and causes telomere shortening, cell cycle arrest and replicative senescence[47]. Moreover, telomeres are less efficient in single-strand break repair than the bulk of the genomic DNA[48] and oxidative stress accelerates telomere loss, whereas antioxidants decelerate it[49]. Mild chronic oxidative stress induced by perturbation of the glutathione redox cycle resulted in accelerated downregulation of telomerase activity, enhanced telomere erosion, and the premature onset of replicative senescence in HUVECs[50]. Homocysteine, a cardiovascular risk factor whose atherogenic effects have been ascribed to increased hydrogen peroxide production[51], also increased the rate of telomere shortening in endothelial cells, and this effect was attenuated in a dose-dependent manner by catalase treatment[52]. On the other hand, prolonged oxidative damage also inhibited telomerase activity and accelerated telomere shortening in vascular smooth muscle cells (VSMCs)[53].

It has been shown that suppression of oxidative stress by Asc2P, an oxidation-resistant derivative of vitamin C, extends the replicative life span by reducing the rate of telomere shortening[54] and reduction of intracellular ROS by antioxidant N-acetylcysteine (NAC) prevented mitochondrial damage and delayed nuclear export of TERT protein, loss of TERT activity and the onset of replicative senescence[55]. The inhibitory effects of oxidant scavenger NAC on telomere attrition and cell death suggest that ROS are important mediators that link mitochondrial dysfunction and telomere shortening and loss, genomic instability, and apoptosis as well. One of the mechanisms for accelerated telomere attrition was demonstrated as formation of 8-oxodG at the GGG-triplet in telomere sequence in response to oxidative damage[56]. Thus, telomeres might also fulfill a function as stress sensors or sentinels for the risk of genomic damage due to low physiological levels of cumulative oxidative damage. While the

Table II. Human studies showing inverse relationship between telomere length and biomarkers of oxidative stress

Biomarkers	Reference
Isoprostanes	18
TBARS (thiobarbituric acid reactive substances, a measure of lipid peroxidation)	25
Aldosterone (proxidant)	28
Oxidative DNA damage	26
8-epi-PGF2α	20
TBARS and protein carbonyl content	21
C-reactive protein (pro-oxidant)	30, 21

telomeres in leukocytes of patients with LHON (Leber hereditary optic neuropathy)- and MELAS-related mitochondriopathies are on average 1.5kb shorter than those of age-matched controls, these respiratory chain disorders are also associated with increased oxidative stress[9]. Recently, it was observed in a number of studies that systemic oxidative stress assessed by various biomarkers (Table II) is associated with shorter telomere lengths. Since increased oxidative stress has been considered as one of the molecular determinants of diseases including diabetes and atherosclerosis, telomere length may provide an additional link between oxidative stress and the predisposition to vascular diseases and metabolic disorders.

Telomere shortening and genes

The search for the causes of hypertension and/or type 2 diabetes has identified several variant genes that may raise blood pressure or blood glucose levels in humans. However, despite the tremendous technological advancements, only modest understanding has been gained about the genetic determinants of these complex human traits. There is also a possibility that the association of shorter telomeres with increased risk of CVDs and/or diabetes has a genetic basis. Several studies have shown that a substantial proportion of the marked inter-individual variation in mean telomere length is genetically determined[57-59]. As telomere length is highly heritable[57], probably X-linked in some cases[39], paternally inherited[60], mapped to a major locus on chromosome12[58] and considered as quantitative trait[61], the role of genetic predisposition to short telomeres in CVDs, diabetes and associated disorders needs further investigations. It is expected that any genetic susceptibility could also be exacerbated or retarded by post-natal effects on telomere length. In addition, future work should also identify functional polymorphisms in telomere-maintenance genes that could serve as independent contributors to risk of type 2 diabetes and cardiovascular diseases.

Conclusions

More basic research and large epidemiological studies are needed to conclusively ascertain whether telomere attrition is an independent cardiometabolic risk factor or a consequence of age-related diseases. Accelerated shortening of telomere length could simply be a surrogate for the chronic oxidative stress and/or inflammation. Similarly more to be studied to examine the efficacy of novel therapeutic strategies aimed at modifying telomere length. There is also much hope in the use of genetically engineered mice exhibiting tissue-specific alterations in telomerase and/or telomere-associated proteins to demonstrate their possible role in the pathogenesis of cardiometabolic diseases. Nevertheless, accelerated telomere shortening appears to be related to 'lifestyle diseases' that accompany certain concomitant metabolic factors such as insulin resistance, obesity, hypernutrition and lack of exercise. It is plausible that the inheritance of shorter telomeres combined with the presence of certain disease-risk factors that determines whether or not subjects progress to an intermediary clinical phenotype, and ultimately suffer a clinical event. This appears to be an outcome in the study of WOSCOPS[23]. Will this accelerated telomere shortening be prevented by tight control of blood glucose, pressure and lipids and/or by caloric restriction and antioxidant supplementation? Since the statin treatment in the WOSCOPS attenuated the increased risk with shorter telomeres[23], it was suggested that telomere length could also identify those individuals who would benefit most from drug intervention. Given that ageing is a multifactorial and highly variable entity and that biological ageing (premature cellular senescence) may alter functional status of several tissues, the use of telomere length provides a new dimension to the study of metabolic and cardiovascular diseases. As more data accumulate regarding telomere dynamics and cellular dysfunction in specific target tissues, one might expect a window of therapeutic opportunities.

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