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AGING AND ENDOTHELIAL PROGENITOR CELL TELOMERE LENGTH IN HEALTHY MEN

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Abstract

Background—Telomere length declines with age in mature endothelial cells and is thought to contribute to endothelial dysfunction and atherogenesis. Bone marrow-derived circulating endothelial progenitor cells (EPCs) are critical to vascular health as they contribute to both reendothelialization and neovascularization. We tested the hypothesis that EPC telomere length decreases with age in healthy adult humans.

Methods—Peripheral blood samples were collected from 40 healthy, non-obese, sedentary men: 12 young (age 21–34 yrs), 12 middle-aged (43–55 yrs) and 16 older (57–68 yrs). Putative EPCs were isolated from peripheral blood mononuclear cells and telomere length was determined using genomic DNA preparation and Southern hybridization techniques.

Results—EPC telomere length (mean base pairs) was ~20% ($P=0.01$) lower in the older (8492 ± 523 bp) compared with the middle-aged (10565 ± 572 bp) and young (10205 ± 501 bp) men. Of note, there was no difference in EPC telomere length between the middle-aged and young men.

Conclusions—These results demonstrate that EPC telomere length declines with age in healthy, sedentary men. Interestingly, telomere length was well preserved in the middle-aged compared with young men, suggesting that EPC telomere shortening occurs after the age of 55 years.

Keywords

aging; endothelial progenitor cells; telomeres

Telomeres are repetitive DNA sequences located at the ends of chromosomes. Their role is to prevent the recognition of chromosomal ends as double stranded breaks and to protect chromosomes from end-to-end fusion and degradation. Gradual telomere shortening occurs with cell replication. However, accelerated telomere attrition results in altered cellular function, increased senescence and reduced survival (1,2). Clinically, telomere shortening in mature endothelial cells as well as white blood cells has been linked to the pathogenesis of atherosclerosis (3,4). Bone marrow-derived circulating endothelial progenitor cells (EPCs) play an vital role in protecting and regenerating the vascular endothelium through their ability to contribute to reendothelialization and neovascularization processes at site of endothelial damage (5). EPC telomere length has been shown to be shorter in patients with coronary artery

disease compared with healthy adults of similar age, potentially contributing to disease risk and burden (6). Ageing is associated with numerical and functional impairment of EPCs which has been linked with endothelial dysfunction, accelerated atherosclerotic disease progression and elevated rates of cardiovascular events (7–9). It has been postulated that telomere attrition may be a factor in age-related EPC dysfunction (10). Currently, however, there are no data regarding the influence of primary ageing on EPC telomere length. Accordingly, we tested the hypothesis that EPC telomere length declines with age in healthy adult humans.

Peripheral blood samples were collected from 40 healthy sedentary men across the adult age-range: 12 young (range: 18–35 years), 12 middle-aged (range: 36–55 years) and 16 older (range: 56–75 years). All subjects were non-obese ($BMI \leq 30.0 \text{ kg/m}^2$ and waist circumference $<102 \text{ cm}$), normotensive ($BP <140/90$), normoglycemic (fasting blood glucose $<6.1 \text{ mmol/L}$), nonsmokers, nonmedicated and free of overt cardiovascular, metabolic and hematologic disease. All subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

Peripheral blood mononuclear cells (PBMNCs) were isolated by ficoll density-gradient centrifugation and plated on human fibronectin coated plates for 48 hours at 37°C as previously described (7). Thereafter, non-adherent cells were removed for DNA extraction. Endothelial phenotype of these cells was confirmed by immunofluorescent staining for the uptake of DiI-ac-LDL and expression of VE-cadherin, von Willebrand factor, CD31, and vascular endothelial growth factor receptor-2 (VEGFR-2) (11–13). In addition, fluorescent-activated cell sorting analysis utilizing endothelial-specific antibodies recognizing cell surface expression of VEGFR-2, CD34, and CD133 (14), was performed in selected samples.

Telomere length of these putative EPCs was determined as described by Middleman and colleagues (15) with minor modifications. Fifteen micrograms of DNA was digested with Hinf I (10U) and Rsa I (10U) at 37°C for 2 hours. The DNA digests were separated on a 0.8% agarose gel overnight. Subsequently, the gel was depurinated with 0.25M HCL, denatured for 30 minutes with 0.5M NaOH/1.5M NaCl and neutralized with a 0.5M Tris-HCL pH 8/1.5M NaCl. DNA was then transferred to a positive charged nylon membrane, UV cross-linked, and hybridized with a ^{32}P -end-labeled (CCCTAA)₃CCC oligonucleotide using Church buffer at 50° overnight. Following hybridization, membranes were washed and exposed to a phosphor-screen for 48 hours. Autoradiographs were scanned on a Typhoon-Trio Imaging system for analysis. Mean EPC telomere length was determined using ImageQuant 5.0 software.

Group differences across the age classifications (young, middle-aged, older) were determined by analysis of variance. When indicated by a significant main effect, post hoc comparisons between the groups using the Newman-Keuls method was performed to identify specific age-group differences. Relations between variables of interest were assessed by linear and stepwise regression analysis. All values are expressed as mean \pm SEM. Statistical significance was set at $P < 0.05$.

Subject characteristics are displayed in the table. The middle-aged and older men demonstrated higher percent body fat and waist circumference compared with young men. Although within clinically normal ranges, systolic blood pressure, plasma concentrations of total cholesterol and low-density lipoprotein cholesterol were significantly higher in older men compared with both the middle-aged and young men. In addition, plasma glucose and oxidized low-density lipoprotein (ox-LDL) were highest in the middle-aged and older men.

Mean EPC telomere length (base pairs) among the groups is shown in the Figure. EPC telomere length was $\sim 20\%$ ($P=0.01$) shorter in the older ($8493 \pm 523 \text{ bp}$) compared with middle-aged ($10565 \pm 572 \text{ bp}$) and young ($10205 \pm 501 \text{ bp}$) men. Of note, there were no differences in EPC

telomere length between the middle-aged and young men. In the overall study population, significant (all $P < 0.05$) univariate correlations were observed with age ($r = -0.36$), BMI ($r = -0.39$), percent body fat ($r = -0.35$), and systolic blood pressure ($r = -0.46$). Stepwise regression analysis identified systolic blood pressure ($R^2 = 0.22$) as the primary determinant of EPC telomere length.

Because systolic blood pressure was highest in the older men and was the primary determinant of EPC telomere length, we compared subgroups of young ($n = 8$; 119/72 mmHg), middle-aged ($n = 8$; 116/76 mmHg) and older ($n = 8$; 121/78 mmHg) subjects with similar systolic blood pressure to address this potential confound. Analogous to the overall population, EPC telomere length was ~22% shorter ($P = 0.01$) in the older (7950 ± 597 bp) compared with middle-aged (10232 ± 437 bp) and young (10318 ± 513 bp) men with similar systolic blood pressure. There was no significant difference in telomere length between the middle-aged and young groups.

The seminal findings of the present study are: 1) telomere length of circulating EPCs decline with age in healthy sedentary men; and 2) the onset of decline appears to be after the age of 55 years. To our knowledge this is the first study to demonstrate an age-related reduction in EPC telomere length in adult humans. Our results extend previous findings demonstrating shortened EPC telomere length with age in other cell types including leukocytes (16) and mature endothelial cells (17) to include circulating progenitor cells.

Numerical and functional impairment of circulating EPCs with age has been attributed to a number of factors including: exhaustion of the pool of stem/progenitor cells in the bone marrow due to chronic vascular injury; reduced mobilization; diminished migratory and adhesion capacity; and dysregulation of EPC differentiation (18). In addition, telomere shortening is thought to be a factor underlying aberrant EPC function with ageing (10). Telomere length may reach a critical threshold resulting in disruption of chromosomal integrity and genomic stability of the ageing cell (19). This, in turn, may trigger a cascade of events that induces cell-cycle arrest, proliferative senescence, and increased apoptotic susceptibility, all of which may lead to a progressive loss in the ability of cells to replicate and function (20).

In the present study, EPC telomere length was significantly shorter (~20%) in older compared with middle-aged and young men providing initial support for the postulate that EPC telomere attrition may contribute to age-related EPC dysfunction. Interestingly, however, EPC telomere shortening does not appear to commence until after the fifth decade of life. EPC telomere length was similar in the middle-aged compared with young men, with both groups demonstrating longer telomeres than the older men. The mechanisms responsible for the reduction in EPC telomere length in men after the age of 55 years are not clear. Although, we studied highly screened clinically healthy men across the adult age range who were non-medicated and free of cardiometabolic diseases, we cannot exclude the possibility that moderate increases in body fatness, blood pressure, metabolic parameters and oxidative stress that commonly occur with advancing age may cumulatively contribute to telomere shortening (21). Moreover, the fact that our subjects were sedentary should not be ignored. Recently, it has been reported that physical inactivity is associated with accelerated telomere attrition in older adult (22).

A number of experimental considerations regarding the present study should be mentioned. Firstly, as with all cross-sectional experimental designs, we cannot ignore the possibility that genetic and/or lifestyle behaviors influenced the results of our study. We attempted to minimize potential lifestyle influences, however, by studying healthy men across the adult age range who were non-medicated, nonsmokers, and not habitually physically active. Secondly, our study pertains only to men, limiting the generalizability of our results. Estrogen has been shown to affect both circulating EPC number and function (23) and previous studies have shown, in a

variety of cell types, that women exhibit less age-dependent telomere erosion than men (24). Thus, the onset and magnitude of decline in EPC telomere length observed in men may differ in women. Thirdly, there are currently no clear consensus criteria for isolating EPCs. It has been recently suggested that peripheral blood mononuclear cells should be cultured for at least 2 weeks to yield true EPCs (25). However, a potential limitation to this approach is phenotypic drift resulting from targeted cell differentiation induced by endothelial-specific culture conditions (26,27); this, in turn, may yield a cellular phenotype that may not be representative of that occurring *in vivo*. Moreover, there are no clinical outcomes data demonstrating that the behavior of these cells following long term culture (>2 weeks) predict any clinical phenomenon. In the present study, we used a well-established panel of endothelial lineage markers for putative EPC characterization in cells whose function *in vitro* has been associated with cardiovascular risk factors and are predictive of clinical events (7–9).

In conclusion, aging is associated with shortened telomere length of circulating EPCs in healthy men. Interestingly, the onset of the decline in telomere length appears to occur after middle age (> 55 years). Future studies are needed to determine whether the reduction in telomere length contributes mechanistically to EPC dysfunction in older adults.

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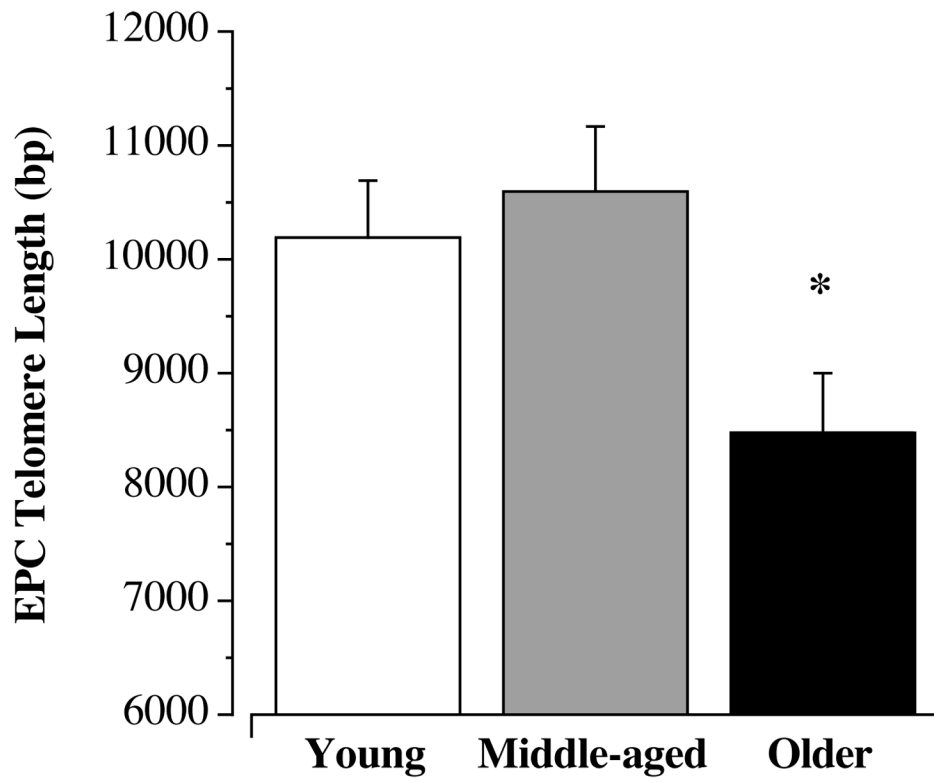


Figure. EPC telomere length (bp) in young, middle-aged and older men. Values are means \pm SEM; * $P = 0.01$ vs. young and middle-aged.

Table

Selected subject characteristics

Variable	Young (n=12)	Middle-aged (n=12)	Older (n=16)
Age, yr	26±1	48±1 [*]	62±1 ^{*†}
Body mass, kg	76.1±2.7	79.8±2.2	81.2±2.4
BMI, kg/m ²	24.2±0.5	24.5±0.6	25.7±0.6
Body fat, %	17.7±1.2	22.4±2.0 [*]	25.1±1.4 [*]
Waist circumference, cm	83.6±1.8	90.0±1.8 [*]	92.5±2.0 [*]
Systolic BP, mmHg	118±2	115±2	128±2 ^{*†}
Diastolic BP, mmHg	70±2	75±2	78±2 [*]
Total cholesterol, mmol/L	4.0±0.2	4.5±0.2	5.4±0.2 ^{*†}
LDL-cholesterol, mmol/L	2.4±0.2	2.7±0.2	3.3±0.2 ^{*†}
HDL-cholesterol, mmol/L	1.2±0.1	1.1±0.1	1.2±0.1
Triglycerides, mmol/L	1.0±0.1	1.4±0.1 [*]	1.3±0.1
Glucose, mmol/L	4.7±0.1	5.1±0.1 [*]	5.3±0.1 [*]
Insulin, pmol/L	36.6±4.1	50.0±5.7	43.4±4.8
ox-LDL, U/L	54.9±4.7	76.1±4.6 [*]	77.7±6.0 [*]

Values are mean ± SEM. BMI: body mass index; BP: blood pressure; LDL: low-density lipoprotein; HDL: high density lipoprotein.

* P < 0.05 vs. young

† P < 0.05 vs. middle-aged.