View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Public Research Access Institutional Repository and Information Exchange

South Dakota State University

Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

Health and Nutritional Sciences Faculty Publications

Health and Nutritional Sciences

1-1-2009

Aging and Endothelial Progenitor Cell Telomere Length in Healthy Men

Erich J Kushner

Gary P. Van Guilder
gary.vanguilder@sdstate.edu

Owen J Maceneaney

Jennifer N Cech

Brian L Stauffer

See next page for additional authors

Follow this and additional works at: http://openprairie.sdstate.edu/hns_pubs

Recommended Citation

Kushner, Erich J; Van Guilder, Gary P.; Maceneaney, Owen J; Cech, Jennifer N; Stauffer, Brian L; and DeSouza, Christopher A, "Aging and Endothelial Progenitor Cell Telomere Length in Healthy Men" (2009). *Health and Nutritional Sciences Faculty Publications*. 62.
http://openprairie.sdstate.edu/hns_pubs/62

This Article is brought to you for free and open access by the Health and Nutritional Sciences at Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Health and Nutritional Sciences Faculty Publications by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact michael.biondo@sdstate.edu.

Authors
Erich J Kushner, Gary P. Van Guilder, Owen J Maceneaney, Jennifer N Cech, Brian L Stauffer, and Christopher A DeSouza

This article is available at Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange: http://openprairie.sdstate.edu/hns_pubs/62

NIH Public Access

Author Manuscript

Clin Chem Lab Med. Author manuscript; available in PMC 2010 January 1.

Published in final edited form as:

Clin Chem Lab Med. 2009 ; 47(1): 47–50. doi:10.1515/CCLM.2009.016.

AGING AND ENDOTHELIAL PROGENITOR CELL TELOMERE LENGTH IN HEALTHY MEN

Erich Kushner[1], **Gary P. Van Guilder**[1], **Owen J. MacEneaney**[1], **Jennifer N. Cech**[1], **Brian L. Stauffer**[1,2,3], and **Christopher A. DeSouza**[1,2]

1*Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, Boulder, Colorado*

2*Department of Medicine, University of Colorado at Denver, Denver, Colorado*

3*Denver Health Medical Center, Denver, Colorado*

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

Correspondence: Christopher DeSouza, Ph.D., Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, 354 UCB, Boulder, CO 80309, TEL: (303) 492-2988, FAX: (303) 492-6778, e-mail: desouzac@colorado.edu.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

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 ≤ 30.0 kg/m^2 and waist circumference <102 cm), normotensive (BP <140/90), normoglycemic (fasting blood glucose <6.1 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 theses cells was confirmed by immunofluorescent staining for the uptake of DiL-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 ± 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 ~20% (P=0.01) shorter in the older (8493±523 bp) compared with middle-aged (10565±572 bp) and young (10205±501 bp) men. Of note, there were no differences in EPC

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

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 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.

Acknowledgments

We would like to thank all of the subjects who participated in the study as well as Jared Greiner and Yoli Casas for their technical assistance and Arthur Zaug for his expert assistance with the telomere length methodology. This study was supported by National Institutes of Health Awards HL076434, HL077450 and RR00051 as well as American Heart Association Award 0555678Z.

REFERENCES

1. Edo MD, Andres V. Aging, telomeres, and atherosclerosis. Cardiovasc Res 2005 May 1;66(2):213–221. [PubMed: 15820190]
2. Artandi SE. Telomeres, telomerase, and human disease. N Engl J Med 2006 Sep 21;355(12):1195–1197. [PubMed: 16990382]
3. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, et al. Telomere shortening in human coronary artery diseases. Arterioscler Thromb Vasc Biol 2004 Mar;24(3):546–550. [PubMed: 14726417]
4. Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. Lancet 2001 Aug 11;358(9280):472–473. [PubMed: 11513915]
5. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res 2004 Aug 20;95(4):343–353. [PubMed: 15321944]
6. Satoh M, Ishikawa Y, Takahashi Y, Tomonori I, Minami Y, Nakamura M. Association between oxidative DNA damage and telomere shortening in circulating endothelial progenitor cells obtained from metabolic syndrome patients with coronary artery disease. Atherosclerosis 2008;198:347–353. [PubMed: 17983621]
7. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003 Feb 13;348(7):593–600. [PubMed: 12584367]
8. Kunz GA, Liang G, Cuculi F, Gregg D, Vata KC, Shaw LK, et al. Circulating endothelial progenitor cells predict coronary artery disease severity. Am Heart J 2006 Jul;152(1):190–195. [PubMed: 16824855]
9. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005 Sep 8;353(10):999–1007. [PubMed: 16148285]
10. Dimmeler S, Vasa-Nicotera M. Aging of progenitor cells: limitation for regenerative capacity? J Am Coll Cardiol 2003 Dec 17;42(12):2081–2082. [PubMed: 14680730]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

11. Fujii H, Li SH, Szmitko PE, Fedak PW, Verma S. C-reactive protein alters antioxidant defenses and promotes apoptosis in endothelial progenitor cells. Arterioscler Thromb Vasc Biol 2006 Nov 26;11:2476–2482. [PubMed: 16931792]
12. Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation 2003 Mar 4;107(8): 1164–1169. [PubMed: 12615796]
13. Verma S, Kuliszewski MA, Li SH, Szmitko PE, Zucco L, Wang CH, et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. Circulation 2004 May 4;109 (17):2058–2067. [PubMed: 15078802]
14. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000 Feb 1;95(3):952–958. [PubMed: 10648408]
15. Middleman EJ, Choi J, Venteicher AS, Cheung P, Artandi SE. Regulation of cellular immortalization and steady-state levels of the telomerase reverse transcriptase through its carboxy-terminal domain. Mol Cell Biol 2006 Mar;26(6):2146–2159. [PubMed: 16507993]
16. Bekaert S, De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Langlois M, et al. Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. Aging Cell 2007 Oct;6(5):639–647. [PubMed: 17874998]
17. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. Proc Natl Acad Sci U S A 1995 Nov 21;92(24):11190–11194. [PubMed: 7479963]
18. Dimmeler S, Zeiher AM. Vascular repair by circulating endothelial progenitor cells: the missing link in atherosclerosis? J Mol Med 2004 Oct;82(10):671–677. [PubMed: 15322703]
19. Shawi MAC. Telomerase, senescence and ageing. Mechanisms of Ageing and Development 2008;129:3–10. [PubMed: 18215413]
20. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol 2007 Oct;3(10):640–649. [PubMed: 17876321]
21. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. Aging Cell 2006 Aug;5(4):325–330. [PubMed: 16913878]
22. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, et al. The association between physical activity in leisure time and leukocyte telomere length. Arch Intern Med 2008 Jan 28;168(2):154–158. [PubMed: 18227361]
23. Strehlow K, Werner N, Berweiler J, Link A, Dirnagl U, Priller J, et al. Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. Circulation 2003 Jun 24;107(24):3059–3065. [PubMed: 12810616]
24. Cherif H, Tarry JL, Ozanne SE, Hales CN. Ageing and telomeres: a study into organ- and gender-specific telomere shortening. Nucleic Acids Res 2003 Mar 1;31(5):1576–1583. [PubMed: 12595567]
25. Fadini GP, Baesso I, Albiero M, Sartore S, Agostini C, Avogaro A. Technical notes on endothelial progenitor cells: ways to escape from the knowledge plateau. Atherosclerosis 2008 Apr;197(2):496–503. [PubMed: 18249408]
26. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22(3):377–384. [PubMed: 15153614]
27. Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 2004 Feb 10;109(5):656–663. [PubMed: 14734516]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

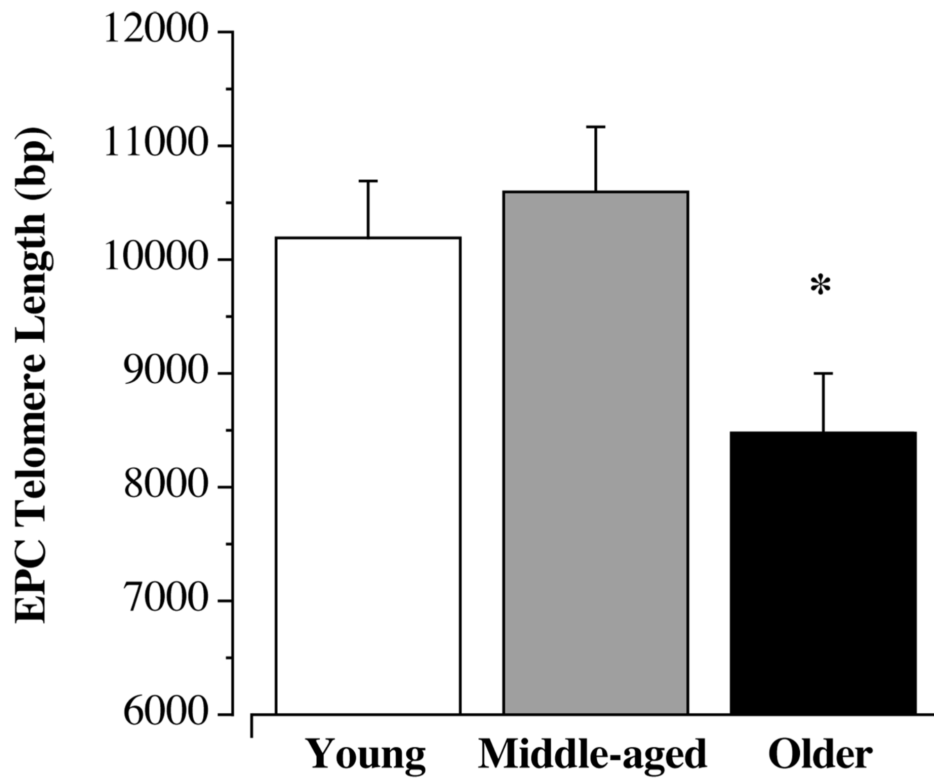

Figure.
EPC telomere length (bp) in young, middle-aged and older men. Values are means ± SEM; *P =0.01 vs. young and middle-aged.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

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±00.5	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.