Human aging and CD31\(^+\) T-cell number, migration, apoptotic susceptibility, and telomere length

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Kushner EJ, Weil BR, MacEneaney OJ, Morgan RG, Mestek ML, Van Guilder GP, Diehl KJ, Stauffer BL, DeSouza CA. Human aging and CD31\(^+\) T-cell number, migration, apoptotic susceptibility, and telomere length. J Appl Physiol 109: 1756–1761, 2010. First published September 23, 2010; doi:10.1152/japplphysiol.00601.2010.—CD31\(^+\) T cells, or so-called “angiogenic T cells,” have been shown to demonstrate vasculo-protective and neovascularogenic qualities. The influence of age on CD31\(^+\) T-cell number and function is unclear. We tested the hypothesis that circulating CD31\(^+\) T-cell number and migratory capacity are reduced, apoptotic susceptibility is heightened, and telomere length is shortened with advancing age in adult humans. Thirty-six healthy, sedentary men were studied: 12 young (25 ± 1 yr), 12 middle aged (46 ± 1 yr), and 12 older (64 ± 2 yr). CD31\(^+\) T cells were isolated from peripheral blood samples by magnetic-activated cell sorting. The number of circulating CD31\(^+\) T cells (fluorescence-activated cell sorting analysis) was lower (P < 0.01) in older (24% of CD3\(^+\) cells) compared with middle-aged (38% of CD3\(^+\) cells) and young (40% of CD3\(^+\) cells) men. Migration (Boyden chamber) to both VEGF and stromal cell-derived factor-1α was markedly blunted (P < 0.05) in cells harvested from middle-aged [306.1 ± 45 and 305.6 ± 46 arbitrary units (AU), respectively] and older (231 ± 65 and 235 ± 62 AU, respectively) compared with young (525 ± 60 and 570 ± 62 AU, respectively) men. CD31\(^+\) T cells from middle-aged and older men demonstrated greater apoptotic susceptibility, as staurosporine-stimulated intracellular caspase-3 activation was ~40% higher (P < 0.05) than young. There was a progressive age-related decline in CD31\(^+\) T-cell telomere length (young: 10,706 ± 220 bp; middle-aged: 10,179 ± 251 bp; and older: 9,324 ± 192 bp). Numerical and functional impairments in this unique T-cell subpopulation may contribute to diminished angiogenic potential and greater cardiovascular risk with advancing age.

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CD31 or platelet/endothelial cell adhesion molecule (PECAM) is a glycoprotein present on the surface of immune cells including platelets, monocytes, and granulocytes (4, 38). The CD31 receptor glycoplin is composed of six extracellular immunoglobulin repeats followed by a transmembrane domain and two cytosolic immunoreceptor tyrosine inhibitory motifs that carry a diverse array of signaling consequences known to be involved in cell phenotype determination, gene expression, and cell cycle (29, 30, 36). The unique structural homology of CD31’s extracellular region is responsible for facilitating numerous heterophilic and homophilic binding interactions between differing vascular tissues (10, 16). For example, CD31 is highly expressed in the border junctions of endothelial cells where it plays a role in endothelial cell motility and regulating leukocyte transendothelial migration (31). In CD31 knockout mice, endothelial cell motility and filopodia formation as well as neovascularization are markedly impaired (5).

Recently, Hur et al. (20) demonstrated that administration of CD31\(^+\) T cells to the ischemic hindlimb of Nude mice significantly enhanced limb perfusion to a greater extent than CD31\(^+\) T cells. This was the first investigation to show that CD31\(^+\) T cells aid in vessel development. In addition, our group (24) recently showed that human CD31\(^+\) T cells possess higher migratory capacity to the proangiogenic compounds stromal cell-derived factor-1α and vascular-endothelial growth factor (VEGF) as well as elevated secretion of interleukin-8, matrix metalloproteinase-9, and granulocyte-colony stimulating factor compared with CD31\(^−\) T cells. Collectively, these findings indicate that CD31\(^+\) T cells are a distinct T-cell subpopulation with a vascular phenotype involved in vessel growth and repair.

Aging is an independent risk factor for the development of cardiovascular diseases and stroke. Diminished endogenous repair capacity is believed to contribute to the etiology of atherosclerotic disease with advancing age (13, 19). Indeed, decreased bioavailability and/or function of reparative factors is linked with a number of age-related pathologies, such as hypertension, diabetes, and obesity (1, 28, 35). Lower circulating CD31\(^+\) T-cell number is associated with vascular disease severity in both animal and human models. Caligiuri et al. (3) demonstrated that reduced circulating CD31\(^+\) T-cell number is an independent predictor of plaque thrombosis in apoE knockout mice, whereas, in humans, low circulating CD31\(^+\) T-cell number is inversely correlated with atherosclerotic aortic abdominal aneurysm surface area (4). Currently, there is a dearth of information regarding the influence of cardiovascular risk factors, such as age, on CD31\(^+\) T-cell biology. Impaired CD31\(^+\) T-cell function may contribute, in part, to increased atherosclerotic disease risk with advancing age. Accordingly, we tested the hypotheses that aging is associated with reduced circulating CD31\(^+\) T-cell number and migratory capacity, increased apoptotic susceptibility, shorter telomere length, and diminished telomerase activity.

METHODS

Subjects. Peripheral blood samples were obtained from 36 healthy adults: 12 young (20–35 yr), 12 middle-aged (36–55 yr), and 12 older (56–75 yr) men. All subjects were sedentary (< 3 days of moderate exercise/wk for at least 1 year before the study), nonsmokers, normotensive (arterial blood pressure ≤ 140/90 mmHg), nonmedicated, and
free of overt cardiovascular and metabolic disease, as assessed by medical history, physical examination, and fasting blood chemistries. Men over the age of 40 yr were further evaluated for clinical evidence of coronary artery disease with electrocardiogram and blood pressure measurements at rest and during incremental exercise performed to exhaustion. The study was reviewed and approved by the University of Colorado at Boulder Institutional Review Board. Before participation, all of the subjects provided written informed consent according to the guidelines of the University of Colorado at Boulder.

**Body composition.** Body mass was measured to the nearest 0.1 kg using a medical beam balance. Percent body fat was determined by dual energy X-ray absorptiometry (Lunar, Madison, WI). Body mass index was calculated as weight (kg) divided by height (m) squared. Minimal waist circumference was measured according to published guidelines (27).

**Maximal oxygen consumption.** To assess aerobic fitness subjects performed incremental treadmill exercise using a modified Balke protocol. Maximal oxygen consumption ($\dot{V}O_2\text{max}$) was measured using on-line computer-assisted open circuit spirometry as previously described (11). In addition, heart rate was measured throughout the protocol and the total exercise time to exhaustion was recorded.

**Magnetic-activated cell sorting.** Peripheral blood mononuclear cells were isolated after an overnight fast from blood by Ficoll density-gradient centrifugation as previously described by our laboratory (24). Following isolation, cells were resuspended in automated magnetic activated cell sorting (MACS) running buffer (Miltenyi Biotec, Bergisch Gladbach, Germany) and CD3+ cells, a general identifier of T cells, were isolated via negative selection using a Pan T cell isolation kit II (Miltenyi Biotec). This procedure excludes cells expressing CD14, CD16, CD19, CD36, CD123, and glycoporphin A. Thereafter, CD3+/CD31+ T cells were positively isolated by incubating with antibodies against CD31-PE and then anti-PE microbeads. The purity of the CD3+CD31+ T cells isolated by this method was verified to be greater than ~90% by flow cytometry. MACS isolated cells were used for migration, apoptosis, telomere length, and telomerase assays.

**Fluorescence-activated cell sorting.** Ficoll-isolated peripheral blood mononuclear cells (2.0×10^6) were incubated at 4°C for 30 min with antibodies against PC7-conjugated CD3 (BD Biosciences, San Jose, CA) and APC-conjugated CD31 (BD Biosciences). Nonviable cells were excluded with propidium iodide. Samples were gated first for CD3 and then analyzed for those events positive for CD31 and presented as percentage of total viable cells. All samples were analyzed using a FC500 flow cytometer (Beckman Coulter, Fullerton, CA), and the data were analyzed using CXP software.

**Migration assay.** The migratory capacity of CD31+ T cells was assessed using a modified Boyden chamber technique, as previously described by our laboratory (19). Briefly, 4×10^5 cells were resuspended in culture medium consisting of RPMI 1640 (CellGro; Mediatech, Manassas, VA), penicillin (100 U/ml), and streptomycin (100 μg/ml), and then plated in the upper buffer chamber of a 24-well modified Boyden chamber coated with fibronectin (FluoroBlok; BD Biosciences). The upper chamber was placed in the lower chamber containing culture medium supplemented with either VEGF (R&D Systems, Minneapolis, MN; 2 ng/ml) or stromal cell-derived factor-1α (SDF-1α; Sigma-Aldrich, St Louis, MO; 20 ng/ml) for 22 h at 37°C. Cells were then labeled with calcein AM (Invitrogen, Carlsbad, CA), and the relative fluorescence was determined in triplicate.

**Apoptosis: caspase-3 and cytochrome c.** Apoptotic tendency of CD31+ T cells was assessed by determining intracellular active caspase-3 and cytochrome c concentrations. Cells designated for caspase-3 analysis were incubated with staurosporine (1 μM) for 3 h. Thereafter, CD31+ T cells were incubated with biotin-ZVAD-fmk (10 μM) for 1 h and lysed in extraction buffer containing leupeptin (25 ng/ml), pepstatin (25 ng/ml), aprotinin (3 μM), and PMSF (100 μM). Active caspase-3 concentrations and cytochrome c concentrations were determined by enzyme immunoassay (R&D Systems).

**Mean telomere restriction fragment length.** Genomic DNA from CD3+/CD31+ T cells plated overnight was prepared using a commercially available DNA extraction kit (Puregene, Valencia, CA), and Southern hybridizations were performed as described previously by our laboratory (25). Briefly, 15 μg of DNA were digested with HindIII (10 U) and RsaI (10 U) (New England Biolabs, Ipswich, MA) at 37°C for 2 h and separated alongside λ HindIII ladder on a 0.8% agarose gel for 16 h at 75 V. Subsequently, the gel was depurinated with 0.25 M HCl, denatured for 30 min with 0.5 M NaOH/1.5 M NaCl, and neutralized for 30 min with 0.5 M Tris-HCl pH 8.1/1.5 M NaCl. DNA was then transferred to Hybond-N+ membrane (Amersham Pharmacia, GE Healthcare, Piscataway, NJ), ultraviolet crosslinked, and hybridized to a 32P-end-labeled (CCCTAA)3CCC oligonucleotide at 50°C overnight in Church buffer. Following hybridization, membranes were washed three times in 0.1% SSC and 0.1% SDS solution at 45°C for 15 min. Autoradiographs were acquired with a PhosphorImager (Typhoon Trio; GE Healthcare) and analyzed with ImageQuant software (v5.2; Molecular Dynamics, GE Healthcare). Telomere length was estimated at the peak positions of the hybridization signals. The mean telomere length was calculated with a two-order linear regression equation using the λ HindIII standard on each gel and the peak position (visually determined) of the hybridization signal (mean distribution) for each individual.

**Telomerase activity.** To stimulate telomerase activity, CD31+ T cells were incubated with activated iBeads (T-cell activation/expansion kit; Miltenyi Biotec) with a 1:2 bead to cell ratio for 24 h following MACS isolation. Thereafter, beads were removed and cells were washed twice with cold PBS on a MACS IMG Separator (Miltenyi Biotec). A telomerase-repeat amplification protocol assay was used for quantitative analysis of telomerase activity (Telo TAGGG PCR ELISA™; Roche Molecular Biochemicals).

**Statistical analysis.** Differences in migration, caspase-3 levels, cytochrome c concentrations, mean telomere restriction fragment length, and telomerase activity were determined by ANOVA. 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 means ± SE. Statistical significance was set at P < 0.05.

## RESULTS

**Subjects.** Selected subject characteristics are presented in Table 1. There were no differences among the groups in body mass index, high-density lipoprotein, and glucose concentrations. Although within clinically normal ranges, the middle-aged and older men demonstrated higher (P < 0.05) body mass, body fat percentage, waist circumference, resting diastolic blood pressure, total cholesterol, and low-density lipoprotein-cholesterol than young controls.

**Fluorescence-activated cell sorting.** There were no age-related differences in the circulating number of CD3+ T cells. However, the number of dual positive CD3+/CD31+ cells was lower (P < 0.01) in the older (24% of CD3+ cells) compared with middle-aged (38% of CD3+ cells) and young (40% of CD3+ cells) men (Fig. 1). The number of CD3+CD31+ cells was not significantly different between the middle-aged and young men. Age was the only univariate correlate of circulating CD31+ T-cell number in the overall study population (r = −0.39; P < 0.05; Fig. 1).

**Cell migration.** Migratory capacity of CD31+ T cells in response to both VEGF and SDF-1α was significantly blunted with age (Fig. 2). The migratory capacity of CD31+ T cells to VEGF and SDF-1α from middle-aged [306.1 ± 44.7 and 305.6 ±
Table 1. Selected subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Men</th>
<th>Middle-Aged Men</th>
<th>Older Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}O_2$ max, l/min</td>
<td>3.9 ± 0.5</td>
<td>2.8 ± 0.2</td>
<td>2.5 ± 1</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>25 ± 1</td>
<td>24.0 ± 0.5</td>
<td>23.0 ± 1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>76.6</td>
<td>85.0 ± 3.3*</td>
<td>82.3 ± 3.7</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>83.5 ± 1.5</td>
<td>94.1 ± 3.4*</td>
<td>94.1 ± 3.9*</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>116 ± 2</td>
<td>120 ± 2</td>
<td>125 ± 2*</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>66 ± 2</td>
<td>80 ± 2*</td>
<td>79 ± 2*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.6</td>
<td>4.9 ± 0.1</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.9</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.2*†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.4 ± 0.2</td>
<td>3.2 ± 0.3*</td>
<td>2.8 ± 0.1*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.0 ± 0.2</td>
<td>4.7 ± 0.2*</td>
<td>4.6 ± 0.2*</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>29.7 ± 3.0</td>
<td>54.1 ± 7.7</td>
<td>66.8 ± 15.1*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.0 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>2.2 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; BP, blood pressure; $\dot{V}O_2$ max, maximal oxygen consumption; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance. *$P < 0.05$ vs. young men. †$P < 0.05$ vs. middle-aged men.

There was a progressive decline in telomere length with age. There were no differences in basal intracellular active caspase-3 concentrations between groups. However, staurosporine-stimulated intracellular active caspase-3 concentrations were ~35% higher ($P < 0.05$) in middle-aged (8.7 ± 1.2 ng/ml) and older (9.0 ± 1.3 ng/ml) compared with young (5.5 ± 1.0 ng/ml) men (Fig. 3). There was no statistical difference in staurosporine-stimulated caspase-3 concentrations between middle-aged and older men. Age was the only significant correlate of stimulated active caspase-3 concentrations ($r = 0.37; P < 0.05$). In a subpopulation of men (7 young, 7 middle aged, and 6 older), there were no statistical differences in basal or staurosporine-stimulated cytochrome c levels between young (19.6 ± 3.7 and 28.9 ± 5.6 ng/ml), middle-aged (13.7 ± 1.3 and 25.6 ± 2.5 ng/ml), and older (15.7 ± 1.6 and 21.8 ± 3.6 ng/ml) groups.

Telomere length and telomerase activity. CD31$^+$ T-cell telomere length (base pairs) among the groups is shown in Fig. 4. There was a progressive decline in telomere length with age. CD31$^+$ T-cell telomere length was markedly shorter ($P < 0.05$) in the older (9.324 ± 192 bp) compared with middle-aged (10.179 ± 252 bp) and young (10.706 ± 220 bp) men.

Fig. 1. A: number of CD31$^+$ T cells expressed as a percentage of CD3$^+$ T cells in young, middle-aged, and older men. B: correlation between CD31$^+$ cells and age. Values are means ± SE. *$P < 0.05$ vs. young men.

Fig. 2. CD31$^+$ T-cell migration to the chemotactic VEGF (A) and stromal cell-derived factor-1α (SDF-1α) (B) in young, middle-aged, and older men. Values are means ± SE. AU, arbitrary units. *$P < 0.05$ vs. young men.

triglycerides ($r = -0.39$ and $-0.47$) for both VEGF and SDF-1α migration. Stepwise regression analysis revealed that body fat percentage was the primary determinant of migratory capacity to both VEGF ($R^2 = 0.26$) and SDF-1α ($R^2 = 0.32$), in the overall study population.
Additionally, telomere length was significantly shorter in the middle aged than young men. In the overall study population, significant (all $P < 0.05$) univariate correlations were observed between telomere length and age ($r = -0.55$), diastolic blood pressure ($r = -0.39$), and triglycerides ($r = -0.58$). Stepwise regression analysis identified triglycerides ($R^2 = 0.34$) as the primary determinant of CD31$^+$ T-cell telomere length accounting for 34% of the variability. Telomerase activity was markedly blunted (~60%, $P < 0.01$ for both) in both the middle-aged (0.31 ± 0.12 AU) and older (0.38 ± 0.14 AU) compared with young (0.94 ± 0.21 AU) men (Fig. 5). A modest correlation was observed between telomere length and telomerase activity ($r = 0.38$, $P < 0.05$).

**DISCUSSION**

The seminal findings of the present study are that aging in healthy adult men is associated with a reduction in circulating CD31$^+$ T-cell number, migratory capacity, telomere length, and telomerase activity and an increase in apoptotic susceptibility. Given the favorable vascular qualities attributed to CD31$^+$ T cells, numerical and functional impairments in this unique T-cell subpopulation may contribute to the greater cardiovascular risk with advancing age.

Peripheral expansion of thymocytes allows for the maintenance of T-cell number with age; however, circulating T-cell subpopulations, such as CD4$^+$/CD45RA$^+$ T cells, have been reported to be drastically altered (15). In the present study, we observed no difference in circulating CD3$^+$ T-cell number with age; however, the number of CD3$^+$/CD31$^+$ T cells was ~40% lower in older compared with middle-aged and young men. Interestingly, the number of CD31$^+$ T cells was well preserved in the middle-aged compared with young men, suggesting that the decline in CD31$^+$ T cells occurs after the age of 55 yr. These findings confirm and extend the results of Hur et al. (20) who reported a significant inverse correlation ($r = -0.70$) between age and CD31$^+$ T-cell number in a comparable population of healthy men. Although the reasons for the decline in circulating CD31$^+$ T-cell number in older adults are not clear, the loss of cells involved in vascular repair may have deleterious consequences (4). It is important to note that all the men studied were sedentary; it would be of interest to determine whether habitual physical activity can prevent and/or reverse the age-related loss in circulating CD31$^+$ T-cell number.

The ability to recognize and home to chemokines is paramount for a prompt and effective endogenous repair response (13). Two critically important chemokines involved in vascular repair and homeostasis are VEGF and SDF-1$\alpha$. VEGF has been deemed the “master-regulator” of angiogenesis due to its role in endothelial proliferation and initiation of vessel sprouting (7, 8). SDF-1$\alpha$ is an important agent in CXCR4-dependent hematopoietic cell migration and cell growth (42). A novel finding of the present study was that CD31$^+$ T cells from both middle-aged and older men demonstrated significantly lower (~60%) migration to VEGF and SDF-1$\alpha$ than cells from young men. In contrast to CD31$^+$ T-cell number, migratory capacity declined precipitously from the young to the middle-aged men and was not different between the middle-aged and older men. This temporal pattern is similar to that previously reported for endothelial progenitor cells (19), supporting the notion that CD31$^+$ T cells comprise part of the circulating vascular progenitor milieu (20). Reduced migratory capacity of circulating CD31$^+$ T cells with age may seriously compromise vascular repair processes and contribute to poor clinical outcomes (13). Indeed, deficient migration and thus limited bioavailability of angiogenic cells at the site of vascular injury and damage have been shown to hinder angiogenesis and neovascularization (13).
Apoptosis or programmed cell death is an intrinsic cellular signaling cascade that is fundamental in organismal development and removal of dysfunctional/stressed cells (2, 17, 23). Age-related activation of apoptotic pathways can lead to premature cellular death and has been implicated in the pathophysiology of cardiovascular disease (40). Herein, we demonstrate that intracellular active caspase-3 concentrations in response to the apoptotic stimulus staurosporine were markedly higher (~35%, P < 0.05) in CD31+ T cells from middle-aged and older compared with young men. Caspase-3 is a cysteine-aspartic acid peptidase that plays a central role in the execution phase of apoptosis; its activation leads to cellular degradation and, subsequently, cell death (9). Increased apoptotic susceptibility with age may not only limit the viability of CD31+ T cells in a proapoptotic environment (such as that encountered in the vascular milieu associated with damage and disease) but also may compromise their function. Indeed, elevations in proapoptotic signaling in endothelial cells, for example, are thought to contribute to vessel dysfunction and plaque development (12, 40).

Telomeres are specialized chromatin structures located at the ends of chromosomes that prevent chromosomal ends from end-to-end fusion, instability, and degradation (6). Shortened telomere length and blunted telomerase activity are deleterious age-related modifications that lower a cell’s ability to resist physiological stressors due to activation of cell senescence and apoptotic programs (34, 41). For example, Werner et al. (37) demonstrated that genetic ablation of telomerase activity in mice significantly increased aortic endothelial cell susceptibility to LPS-induced apoptosis. Reduced telomere length and telomerase activity may precede and predispose to cellular dysfunction with age (18). Moreover, endothelial progenitor cells from coronary artery disease patients with the metabolic syndrome have been shown to demonstrate significantly eroded telomere length and telomerase activity compared with those without the metabolic syndrome (33). In the current study, we observed a progressive decline in telomere length with age in CD31+ T cells. The magnitude of the age-related reduction in telomere length (~40 bp/yr) in the present study is consistent with that previously reported in other cell lines (25, 26). Concurrent with the reduction in telomere length, telomerase activity was ~40% lower in CD31+ T cells from middle-aged and older compared with young men. There was a modest correlation between telomere length and telomerase activity in the present study, suggesting a potential biological effect of reduced telomerase activity with age on the changes in telomere length. Interestingly, we also observed a significant correlation between telomere length and migration [to both VEGF (r = 0.42) and SDF-1α (r = 0.45)], suggesting a link between telomere length reduction and CD31+ T-cell function.

The mechanisms responsible for the observed numerical and functional impairments in circulating CD31+ T cells in healthy men are not clear. With respect to the age-related decline in number, although the general peripheral T-cell population was preserved between groups, the reduction in circulating CD31+ T-cell number may be due to thymic involution and the resulting decrease in thymic output of naive T cells (15). One possible explanation for the reduction in migration is less CXCR4 (SDF-1α receptor) and VEGF receptor-2 cell surface expression in CD31+ T cells with age. Diminished receptor expression would limit chemotactic responses to VEGF and SDF-1α, thus impairing migratory capacity. Moreover, several investigations (32, 39) have shown that telomerase activity is positively linked to CXCR4/SDF-1α expression. Therefore, it is possible that the blunted migratory capacity observed in CD31+ T cells from middle-aged and older men is due, at least in part, to reduced telomerase activity. Telomerase activity may also impact apoptotic susceptibility. Dong et al. (14) reported that elevated telomerase activity is associated with Akt phosphorylation, an upstream caspase-3 inhibitor, in endothelial progenitor cells treated with ginkgo biloba extract. Although we did not observe a significant relation between telomerase activity and caspase-3 activity in the present study, we cannot dismiss the possibility (due to our relatively modest sample size and the inherent interpretive causal limitations of correlation analysis) that reduced telomerase activity may contribute to the greater apoptotic susceptibility observed in the CD31+ T cells from the middle-aged and older men. In the present study, migration and telomere length were correlated with body fat percentage and triglycerides, suggesting a potential influence of these factors on the observed main effect of age. Clearly, future studies are needed to delineate the mechanisms responsible for the numeric and functional changes observed in CD31+ T cells with advancing age.

There are at least two limitations of the present study that require consideration. Firstly, due to the cross-sectional study design, we cannot ignore the possibility that genetic and/or lifestyle behaviors influenced the results of our study. In an effort to isolate the primary effects of age, per se, all subjects were free of overt disease, nonmedicated, nonsmokers, and sedentary. Secondly, our results pertain only to men. Considering estrogen has been shown to affect both the number and function of circulating cells associated with vascular repair (21, 22), the onset and magnitude of the age-related decline in CD31+ T-cell number and migratory capacity observed in men may differ in women. Thus the results of this study should be viewed in this context and any extrapolation to women done with caution.

In summary, the results of this study indicate that advancing age is associated with lower circulating number, decreased migratory capacity, elevated apoptotic susceptibility, shorter telomeres, and blunted telomerase activity in CD31+ T cells in healthy men. Given the emerging role of CD31+ T cells in angiogenic and vascular repair processes, age-related impairments in number and function may contribute, in part, to the heightened risk of vascular complications in older men.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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