Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men

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J Appl Physiol 102: 847–852, 2007. First published December 7, 2006; doi:10.1152/japplphysiol.01183.2006. —Numerical and functional impairment of circulating endothelial progenitor cells (EPCs) is thought to contribute to vascular aging and the associated increase in cardiovascular risk. We tested the following hypotheses: 1) EPC clonogenic and migratory capacity decrease progressively with age in healthy, sedentary adult men; and 2) regular aerobic exercise will improve EPC clonogenic and migratory capacity in previously sedentary middle-aged and older men. Peripheral blood samples were collected from 46 healthy sedentary men: 10 young (26 ± 1 yr), 15 middle-aged (47 ± 1 yr), and 21 older (63 ± 1 yr). Mononuclear cells were isolated and preplated for 2 days, and nonadherent cells were further cultured for 7 days to determine EPC colony-forming units. Migratory activity of EPCs was determined using a modified Boyden chamber. Ten sedentary middle-aged and older men (59 ± 3 yr) were studied before and after a 3-mo aerobic exercise intervention. The number of EPC colony-forming units was ~75% lower (P < 0.01) in middle-aged (12 ± 3) and older (8 ± 2) compared with young (40 ± 7) men. There was no difference in colony count between middle-aged and older men. EPC migration (fluorescent units) was significantly reduced in older (453 ± 72) compared with young (813 ± 114) and middle-aged (760 ± 114) men. The exercise intervention increased (P < 0.05) both EPC colony-forming units (10 ± 3 to 22 ± 5) and migratory activity (683 ± 96 to 1,022 ± 123) in previously sedentary middle-aged and older men. These results provide further evidence that aging adversely affects EPC function. Regular aerobic-endurance exercise, however, is an effective lifestyle intervention strategy for improving EPC clonogenic and migratory capacity in middle-aged and older healthy men.

vascular progenitor cells; clonogenic capacity; migratory capacity; cardiovascular disease

CLINICAL INTEREST IN BONE marrow-derived circulating vascular progenitor cells, specifically endothelial progenitor cells (EPCs), has increased due to their importance to reendothelialization and neovascularization processes (9, 42), as well as their emerging role as a biomarker of cardiovascular risk (18, 33, 44, 45). Circulating EPCs home to sites of ischemia and vascular injury as a repair mechanism to denuded or dysfunctional endothelium (8, 47). Indeed, EPC-mediated vascular repair has been shown to be associated with normalization of endothelial function and restoration of blood flow at the site of injury (13, 15). Numerical and functional impairment of EPCs, however, has been linked to endothelial dysfunction (17, 18, 28), increased atherosclerotic disease risk (11, 12, 44), and greater cardiovascular morbidity and mortality (33, 45).

The incidence and prevalence of cardiovascular disease and its clinical consequences increase with advancing age in humans (1). Declines in circulating EPC bioavailability and function have been suggested to contribute etiologically to age-related vascular dysfunction and disease (7, 17, 32). In contrast to aging, regular aerobic exercise is associated with reduced incidence of cardiovascular disease, especially in middle-aged and older adults (3, 4, 26). Although the mechanisms by which exercise confers this cardioprotection have not been fully elucidated, the favorable effects of exercise on vascular health and function are considered to be a major factor. Our laboratory and others have previously reported that habitual aerobic exercise may not only prevent, but can also reverse, age-related reductions in endothelial vasodilator and fibrinolytic function (6, 35). Currently, it is unknown whether regular exercise has similar beneficial effects on functional characteristics of EPCs in healthy middle-aged and older sedentary adults.

Accordingly, we tested the following hypotheses: 1) EPC clonogenic and migratory capacity decrease progressively with age in healthy, sedentary adult men; and 2) regular aerobic exercise will improve EPC clonogenic and migratory capacity in previously sedentary middle-aged and older men. To test these hypotheses, we used a cross-sectional study design to determine the influence of primary aging on EPC colony-forming and migratory capacity followed by an intervention study to determine the effects of moderate aerobic exercise training on these two functional characteristics of EPCs in sedentary middle-aged and older men.

METHODS

Subjects

Forty-six healthy sedentary adults participated in the cross-sectional study: 10 young (22–35 yr), 15 middle-aged (36–55 yr), and 21 older (56–75 yr) men. All subjects were normotensive (arterial blood pressure ≤140/90 mmHg) 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 electrocardiograms and blood pressure at rest and during incremental exercise performed to exhaustion. None of the subjects smoked, were

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taking medications, or performed regular physical exercise for at least 6 mo before the start of the study. Ten middle-aged and older men from the cross-sectional investigation were studied before and after 3 mo of aerobic exercise training. Before participation, all of the 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.

Measurements

Subjects who completed the 3-mo exercise intervention were studied 20–24 h after their last exercise training session to avoid the immediate (acute) effects of exercise while still representing their normal physiological state (i.e., habitually exercising).

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 (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines (24).

Maximal oxygen consumption. To assess aerobic fitness, subjects performed incremental treadmill exercise using a modified Balke protocol. Maximal oxygen consumption (\(\dot{V}O_2\)max) was measured using online computer-assisted open-circuit spirometry as previously described (5). In addition, heart rate was measured throughout the protocol, and the total exercise time to exhaustion was recorded.

Metabolic measurements. Fasting plasma lipid and lipoprotein, glucose, and insulin concentrations were determined using conventional methods by the clinical laboratory affiliated with the General Clinical Research Center.

EPC clonogenic capacity. EPC colony-forming capacity was determined as previously described (18, 19). Briefly, peripheral blood mononuclear cells were isolated by Ficoll density-gradient centrifugation (Histopaque 1077). The recovered cells were then washed (phosphate-buffered saline), resuspended in growth medium [medium 199, supplemented with 20% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 \(\mu g/ml\)], and plated on six-well plates coated with human fibronectin (BD Biosciences) for 48 h. Thereafter, non-adherent cells were collected, and 5 \(\times 10^5\) cells were replated onto 24-well fibronectin-coated plates (BD Biosciences). Cells for each subject were plated in 6 wells, growth medium was changed every 3 days, and the final count of colony-forming units (CFUs) was determined on day 7. CFUs were counted manually in four random wells. Only CFUs consisting of multiple thin, flat cells emanating from a central cluster of rounded cells were counted. These early-outgrowth CFUs are regarded to portray stem cell-like properties (45). Endothelial-cell lineage was confirmed by fluorescent-activated cell sorting (FACS) analysis in selected samples utilizing endothelial-specific antibodies recognizing cell surface expression of vascular endothelial growth factor (VEGF) receptor-2, CD34, and CD133 (25). All FACS analysis was performed by the University of Colorado Cancer Center Flow Cytometry core laboratory.

Migration assay. Migratory capacity was assessed using a modified Boyden chamber technique as previously described by our laboratory (19). Briefly, nonadherent cells (4 \(\times 10^5\)), resulting from the isolation techniques noted above, were resuspended in culture medium, consisting of medium 199, penicillin (100 U/ml), and streptomycin (100 \(\mu g/ml\)), and then placed in the upper 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 and VEGF (2 ng/ml) for 2 h at 37°C. Cells were then labeled with calcein AM (Molecular Probes), and the fluorescence of the migrated cells was determined in triplicate and the mean relative fluorescent units presented.

Exercise Intervention

The 3-mo home-based moderate aerobic exercise-training program utilized in the present study has been previously described in detail by our laboratory (35). Briefly, after orientation to the exercise program, subjects were asked to exercise 5–7 days/wk, 40–50 min/day, at 60–75% of their individual maximum heart rate, as determined during maximal exercise testing. Most subjects walked, but some integrated jogging into their exercise session, as their fitness improved, to maintain their heart rate within the prescribed range. Program adherence was documented every 2 wk from data downloaded directly from heart rate monitors (Polar Electro, Woodbury, NY) and from exercise logs.

Statistical Analysis

The cross-sectional data were analyzed by ANOVA. When indicated by a significant main effect, post hoc test using the Newman-Keuls method was performed to identify significant group differences. Relations between variables of interest were assessed by linear and stepwise regression analysis. Changes in the dependent variables resulting from the exercise intervention were assessed by repeated-measures ANOVA. Analysis of covariance was performed for group differences at baseline with the variable in question serving as the covariate. All data are expressed as means \(\pm\) SE. Statistical significance was set a priori at \(P < 0.05\).

RESULTS

Cross-Sectional Study

Selected subject characteristics are presented in Table 1. Body mass and body composition values tended to be greater in the middle-aged and older groups. \(\dot{V}O_2\)max was significantly higher in the young men compared with both the middle-aged and older sedentary men. There were no differences among the groups in plasma lipid and lipoprotein, glucose, and insulin concentrations. Importantly, covarying for differences in baseline characteristics did not affect the age-related differences observed in the primary outcome variables.

The number of EPC CFUs was \(\sim 70\%\) lower \((P < 0.01)\) in the middle-aged \((12 \pm 3)\) and older \((8 \pm 2)\) groups compared with the young \((40 \pm 7)\) group (Fig. 1A). There was no

| Table 1. Selected subject characteristics of the cross-sectional study |
|-----------------|-----------------|-----------------|
| Variable        | Young \(n = 10\) | Middle Aged \(n = 15\) | Older \(n = 21\) |
| Age, yr         | 26.1\(\pm\)1*   | 47\(\pm\)1*      | 63\(\pm\)1\(\dagger\) |
| Body mass, kg   | 76.4\(\pm\)2.6  | 90.8\(\pm\)4.6*  | 81.7\(\pm\)2.3*   |
| Body fat, %     | 15.5\(\pm\)1.4  | 26.6\(\pm\)2.1*  | 26.2\(\pm\)1.5*   |
| Waist circumference, cm | 84.3\(\pm\)1.8  | 97.0\(\pm\)3.7*  | 93.4\(\pm\)2.1*   |
| BMI, kg/m²      | 24.1\(\pm\)1.3  | 27.8\(\pm\)1.0*  | 26.2\(\pm\)0.6    |
| Systolic BP, mmHg | 115\(\pm\)5    | 120\(\pm\)2      | 122\(\pm\)2      |
| Diastolic BP, mmHg | 67\(\pm\)3    | 81\(\pm\)1*      | 79\(\pm\)1*      |
| \(\dot{V}O_2\)max, ml/kg-l\(^{-1}\)*min\(^{-1}\) | 49.3\(\pm\)1.5  | 37.7\(\pm\)1.8*  | 33.6\(\pm\)1.4*   |
| Total cholesterol, mmol/l | 4.5\(\pm\)0.3  | 4.9\(\pm\)0.2   | 5.1\(\pm\)0.1    |
| HDL-C, mmol/l   | 1.2\(\pm\)0.1  | 1.1\(\pm\)0.1    | 1.3\(\pm\)0.1    |
| LDL-C, mmol/l   | 2.8\(\pm\)0.3  | 3.1\(\pm\)0.1    | 3.2\(\pm\)0.1    |
| Triglycerides, mmol/l | 1.1\(\pm\)0.2  | 1.4\(\pm\)0.2   | 1.2\(\pm\)0.1    |
| Glucose, mmol/l | 4.9\(\pm\)0.2  | 5.1\(\pm\)0.1    | 5.2\(\pm\)0.1    |
| Insulin, pmol/l | 28.4\(\pm\)2.6 | 36.0\(\pm\)6.1  | 29.7\(\pm\)2.7   |

Values are means \(\pm\) SE; \(n\), no. of subjects. BMI, body mass index; BP, blood pressure; \(\dot{V}O_2\)max, maximal oxygen consumption; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol. *\(P < 0.05\) vs. young. †\(P < 0.05\) vs. middle-aged.
significant difference in colony count between the middle-aged and older men. Migratory activity was significantly lower in the older (453 ± 72 relative fluorescent units (RFUs)) compared with the middle-aged (760 ± 14 RFUs) and young (813 ± 114 RFUs) groups. Mean fluorescence was similar between the middle-aged and young men (Fig. 1B).

In the overall study population, the total number of CFUs was related to age ($r = -0.66; P < 0.01$), percent body fat ($r = -0.37; P < 0.01$), $V_O^{2\text{max}}$ ($r = 0.48; P < 0.01$), systolic blood pressure ($r = -0.48, P < 0.01$), diastolic blood pressure ($r = -0.38; P < 0.05$), and LDL cholesterol ($r = -0.36; P < 0.05$). Stepwise regression analysis revealed that age ($R^2 = 0.34$) was the primary determinant of clonogenic capacity. Age was the only significant correlate with EPC migration ($r = -0.34; P < 0.05$).

**Intervention Study**

All 10 middle-aged and older men (59 ± 3 yr) completed the exercise intervention. On average, the men walked 5.0 ± 0.5 days/wk, 48 ± 2 min/day, at an intensity of 67 ± 1% of individually determined maximal heart rate. There were no significant changes in body mass; body composition; blood pressure; heart rate at rest; or plasma lipids, glucose, or insulin concentrations (Table 2). Although $V_O^{2\text{max}}$ did not significantly increase heart rate at a standardized submaximal workload, ~70% of the initial (baseline) $V_O^{2\text{max}}$ was lower and maximal treadmill walking time increased (~30%; $P < 0.05$) in response to exercise training (Table 2).

Regular aerobic exercise resulted in significant increases in both EPC CFUs and migratory capacity. The number of EPC colonies doubled from 10 ± 3 to 22 ± 5, and migratory activity increased by ~50% (from 683 ± 96 to 1,022 ± 123 RFUs) after the exercise training program (Fig. 2). There were no significant correlates of the improvement in colony formation or migratory activity in response to exercise training.

**DISCUSSION**

The main findings of the present study are as follows. First, both EPC clonogenic and migratory capacity decline with age in healthy, sedentary men. Moreover, the onset of decline in EPC clonogenic capacity appears to occur at an earlier age compared with the decline in migratory activity. Second, regular aerobic exercise increases EPC clonogenic and migratory capacity in previously sedentary middle-aged and older men, independent of changes in body mass and composition, cardiometabolic risk profile, or maximal aerobic capacity. To our knowledge, this is the first study to delineate the onset of the age-related decline in EPC clonogenic and migratory activity and demonstrate that moderate aerobic exercise training can improve these functional characteristics of EPCs in middle-aged and older men.

Both experimental (21, 22) and clinical (2, 37) studies have demonstrated the importance of EPCs to vascular homeostasis. EPCs contribute to the reendothelialization and neovascularization of ischemic tissue in coronary and peripheral artery disease (8, 46). The capacity to maintain, repair, or regenerate the endothelial monolayer and restore functional activity is essential to prevent atherosclerotic lesion development and thrombus formation (9, 42). Many of the cardiovascular complications associated with age are due, at least in part, to endothelial damage and/or dysfunction (34, 39, 40). Numerical and functional impairment of EPCs leading to compromised vascular repair are thought to be major factors underlying the development of an atherogenic endothelial phenotype with aging (10, 28, 29). Indeed, EPC clonogenic and migratory

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Training</th>
<th>After Training</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>59 ± 2</td>
<td>83.2 ± 3.5</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>82.8 ± 3.3</td>
<td>25.1 ± 2.1</td>
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<tr>
<td>Body fat, %</td>
<td>26.1 ± 1.8</td>
<td>93.3 ± 3.0</td>
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<tr>
<td>Waist circumference, cm</td>
<td>122 ± 3</td>
<td>78.3 ± 2.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8 ± 0.7</td>
<td>26.8 ± 0.7</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>122 ± 3</td>
<td>121 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>78 ± 3</td>
<td>74.2</td>
</tr>
<tr>
<td>$V_O^{2\text{max}}, \text{ml/kg}^{-1}\text{min}^{-1}$</td>
<td>34.6 ± 2.3</td>
<td>37.1 ± 3.2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
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<td>HDL-C, mmol/l</td>
<td>3.1 ± 0.2</td>
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<td>LDL-C, mmol/l</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.2</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>3.1 ± 0.6</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>10.9 ± 0.4</td>
<td>13.8 ± 1.0*</td>
</tr>
<tr>
<td>Submaximal heart rate, beats/min</td>
<td>155 ± 7</td>
<td>144 ± 6*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. *P < 0.05 vs. before training.
capacity have been shown to be related to both flow-mediated brachial artery reactivity (17, 18) and Framingham risk score in middle-aged and older men. Moreover, EPC colony formation was reported to be a better predictor of endothelial dysfunction than conventional risk factors in this population (18). The results of the present study confirm and significantly extend previous studies demonstrating age-associated reductions in EPC function (17, 18). For example, our finding of reduced EPC migratory response to VEGF in older compared with young men is consistent with those of Heiss et al. (17) and supports the notion that diminished migratory capacity is a hallmark feature of cellular aging (28, 30). The ability of circulating EPCs to migrate is critical for homing to sites of endothelial injury and damage: impaired migratory ability limits the availability of EPCs at the site of injury, hindering repair and neovascularization (8). In addition, the loss of EPC clonogenic capacity observed in our middle-aged and older adults confirms initial correlational observations by Hill et al. (18), suggesting a negative influence of age on EPC differentiation and proliferation (14, 27).

An interesting and novel finding of the present study is that the onset of the age-related decline in EPC clonogenic and migratory capacity was not uniform. The capacity of EPCs to form colonies declined sharply (~60%) after the age of 35 yr and appeared to nadir in middle age because there was no significant difference in colony count between the middle-aged and older men. In contrast, the ability of EPCs to migrate was preserved in middle-aged men compared with the young controls, with the older men demonstrating levels ~40% below both the middle-aged and young subjects. The mechanisms responsible for the age-related reduction in EPC clonogenic and migratory capacity are not clear. The observed temporal difference in the onset of decline in these two functional characteristics tends to argue against a mutual underlying mechanism. It has been suggested that aging may be associated with impaired EPC mobilization and differentiation, telomere attrition, enhanced senescence and apoptotic rates, as well as dysfunctional receptor-dependent signaling pathways (7, 10, 17, 32). Each of these factors independently, or combined, may contribute to the observed differences in the age-related decline in EPC clonogenic and migratory function.

Regular aerobic exercise has been shown to be an extremely effective intervention strategy for improving vascular endothelial function in both healthy and diseased populations (6, 16, 43). The results of our exercise intervention study demonstrate the beneficial effects of moderate aerobic exercise training on EPC function in previously sedentary middle-aged and older men. Specifically, 3 mo of moderate aerobic exercise (primarily walking) resulted in a 120% increase in colony formation and 50% increase in migratory capacity. Of note, the increases in EPC colony number and migration occurred independent of changes in body mass, adiposity, arterial blood pressure, and lipid and lipoproteins, suggesting a direct effect of aerobic exercise on EPC function. Our findings in healthy adult men compliment and extend previous studies demonstrating exercise-induced increases in circulating EPC number in patients with cardiovascular and peripheral vascular disease (23, 31, 36). For example, Steiner and colleagues (36) reported that 12 wk of supervised endurance aerobic exercise training resulted in a threefold increase in circulating EPCs (defined as CD34+/KDR+/CD133+ cells) in patients with cardiovascular risk factors and coronary artery disease. Moreover, the authors reported that the increase in circulating EPCs was strongly and positively associated (r = 0.81) with exercise-induced improvement in flow-mediated brachial reactivity (36). The latter finding suggests that enhanced bioavailability of EPCs may underlie the beneficial effects of habitual aerobic exercise on endothelial function. It is important to emphasize that the improvements in clonogenic and migratory capacity observed in the present study were accomplished with a home-based aerobic exercise program, employing a mode (walking) and intensity (moderate) of exercise that can be safely performed by most sedentary healthy middle-aged and older adults. Greater EPC number and function, resulting in more efficient vascular repair, may contribute to the lower rates of vascular events in middle-aged and older adults who engage in habitual physical activity (3, 4, 26).

It is important to view the results of the present study within the context of our experimental design and the limitations thereof. First, we cannot dismiss the inherent possibility that genetic and/or other lifestyle behaviors influenced the results of our cross-sectional study. We attempted to minimize potential lifestyle influences by studying healthy men across the adult age range who were nonmedicated, were nonsmokers, and did not engage in habitual physical activity. Additionally, in an effort to isolate the primary effects of aging per se, all subjects were free of cardiometabolic risk factors, such as hypertension,
dyslipidemia, and diabetes that are common comorbidities of aging and known to negatively influence EPC clonogenic and migratory activity (18, 28, 44). Second, our results pertain only to men. Given the fact that estrogen has been shown to affect both circulating EPC number and function (38), the onset and magnitude of the age-related decline in EPC colony-forming and migratory capacity observed in men may differ markedly in women. Indeed, our laboratory has recently shown that EPC clonogenic and migratory capacity are significantly higher (~150% and ~40%, respectively) in samples collected from middle-aged women compared with men (20). Finally, the lack of a nonexercising control group is a limitation of our intervention study design. However, our laboratory and others have repeatedly shown that the exercise training program employed in the present study confers important vascular benefits, including improvements in central arterial compliance (41) as well as vascular endothelial vasodilator (6) and fibrinolytic (35) function, in middle-aged and older adults. Thus it is not unreasonable to assume that the observed exercise-induced increase in EPC clonogenic and migratory capacity were indeed main effects of the intervention.

In conclusion, EPC dysfunction resulting in incompetent vascular repair is thought to contribute to the increased risk of atherosclerosis and the prolonged and often complicated recovery from ischemic events in older adults (7, 29). The results of the present study provide further evidence that aging, independent of traditional cardiovascular risk factors, adversely affects EPC clonogenic and migratory function in sedentary men. Importantly, regular aerobic-endurance exercise is an effective lifestyle intervention strategy for improving EPC colony-forming capacity and migratory activity in middle-aged and older healthy men. The clinical importance of the effects of exercise on EPC biology remains to be verified. Nevertheless, greater vascular repair potential may represent an important mechanism underlying the reduced cardiovascular risk observed in middle-aged and older men who exercise regularly.

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