Human Exercise-Induced Circulating Progenitor Cell Mobilization Is Nitric Oxide-Dependent and Is Blunted in South Asian Men


**Objective**—Circulating progenitor cells (CPC) have emerged as potential mediators of vascular repair. In experimental models, CPC mobilization is critically dependent on nitric oxide (NO). South Asian ethnicity is associated with reduced CPC. We assessed CPC mobilization in response to exercise in Asian men and examined the role of NO in CPC mobilization per se.

**Methods and Results**—In 15 healthy, white European men and 15 matched South Asian men, CPC mobilization was assessed during moderate-intensity exercise. Brachial artery flow-mediated vasodilatation was used to assess NO bioavailability. To determine the role of NO in CPC mobilization, identical exercise studies were performed during intravenous separate infusions of saline, the NO synthase inhibitor L-NMMA, and norepinephrine.

Flow-mediated vasodilatation (5.8% ± 0.4% vs 7.9% ± 0.5%; \(P=0.002\)) and CPC mobilization (CD34+/KDR+ 53.2% vs 85.4%; \(P=0.001\); CD133+/CD34+/KDR+ 48.4% vs 73.9%; \(P=0.05\); and CD34+/CD45− 49.3% vs 78.4%; \(P=0.006\)) was blunted in the South Asian group. CPC mobilization correlated with flow-mediated vasodilatation and L-NMMA significantly reduced exercise-induced CPC mobilization (CD34+/KDR+ −3.3% vs 68.4%; CD133+/CD34+/KDR+ −0.7% vs 71.4%; and CD34+/CD45− −30.5% vs 77.8%; all \(P<0.001\)).

**Conclusion**—In humans, NO is critical for CPC mobilization in response to exercise. Reduced NO bioavailability may contribute to imbalance between vascular damage and repair mechanisms in South Asian men. (Arterioscler Thromb Vasc Biol. 2010;30:878-884.)

Key Words: blood cells • endothelium • exercise • nitric oxide

The complications of atherosclerosis are a major global cause of death and disability. Ethnicity has emerged as an important risk factor for the development of atherosclerosis, with people of South Asian ethnicity being at particularly high risk. As a result, young South Asian men are up to 3-times more likely to sustain an acute myocardial infarction than white European men. The mechanisms underlying this increased risk are unclear. An emerging paradigm is that the development of atherosclerosis is critically dependent on a delicate balance between endothelial cell damage and the capacity to repair and regenerate damaged endothelium. Endothelial repair was once thought to be restricted to the migration and proliferation of mature endothelial cells local to the site of injury. However, more recent evidence suggests that circulating progenitor cells (CPC) are mobilized from the bone marrow in response to various cues, home to sites of damage, and contribute to vascular repair. We recently demonstrated that young South Asian men resident in the United Kingdom have endothelial dysfunction and reduced CPC. The mechanisms underlying this finding are unclear. CPC-mediated vascular repair is thought to be critically dependent on their appropriate mobilization into the circulation. Whereas many CPC mobilizing stimuli have been identified in humans, the mechanisms regulating CPC mobilization remain incompletely understood. Experimental studies have suggested that CPC mobilization in response to vascular endothelial growth factor or exercise is critically dependent on nitric oxide (NO) bioavailability.

A number of studies in humans have shown that exercise-induced CPC mobilization correlates with surro-
gates of NO bioavailability, but definitive evidence of a pivotal role for NO in CPC mobilization in humans is lacking. We performed a series of studies to ascertain whether exercise-induced CPC mobilization is impaired in healthy South Asian men and whether perturbation of NO bioavailability per se is important in human EPC mobilization.

**Subjects and Methods**

Thirty healthy, nonsmoking, male subjects (15 white European, 15 South Asian) were recruited; classification of ethnicity was subject-defined as previously reported. All subjects were screened for cardiovascular abnormalities, which would preclude maximal exercise testing according to the European Society of Cardiology guidelines. Fasting insulin concentration was quantified using enzyme-linked immunosorbent assay (Merckodia); and homeostasis index of insulin resistance, an index of insulin resistance, was calculated with corresponding fasting glucose data using the formula homeostasis index of insulin resistance: fasting insulin × fasting glucose/22.5. Studies were performed with appropriate local and institutional ethics committee approval and all subjects provided informed consent.

**Assessment of Maximum Aerobic Capacity and Lactate Threshold**

Exercise was performed on an electromagnetically braked cycle ergometer (Excalibur Sport; Lode BV) with continuous measurement of cardiopulmonary variables: heart rate and electrocardiography by 12-lead ECG (Quest Stress; Burdick); arterial oxygen saturation by pulse oximetry (Biox 3745; Ohmeda); expired gas tensions by mass spectrometry (MSX: Morgan Medical); and inspired and expired ventilation by turbinometry (Volume Transducer; Interface Associates). Pulmonary gas exchange (eg, oxygen uptake; VO2) and ventilatory (eg, ventilation; V̇E) variables were measured breath-by-breath using the algorithms of Beaver et al. The subjects were familiarized with the testing environment and procedures during an initial exercise test. Subsequently, subjects performed a ramp-incremental exercise test (15 watts/ min) to the limit of tolerance to and from baseline cycling of 20 watts. This allowed maximum oxygen uptake (VO2max) and lactate threshold to be estimated, with the latter using the V-slope method and supporting ventilatory and pulmonary gas exchange criteria.

**Assessment of Brachial Artery Endothelium-Dependent Vasodilatation**

Endothelial-dependent and endothelial-independent vasodilatation of the brachial artery were assessed as previously described. In brief, the change in brachial artery diameter was measured from B-mode ultrasound images using a standard 7-MHz linear array transducer and Acuson Aspen system with automated edge detection (Brachial Analysis System; Medical Imaging Applications).

Subjects lay supine for 30 minutes before the first scan and remained so throughout the study. The brachial artery was scanned in a longitudinal section 2 to 15 cm above the elbow, and the center of the artery was identified when the clearest picture of the anterior and posterior wall layers was obtained. When a satisfactory transducer position was found, the skin was marked and both the probe and arm were immobilized throughout the study. Images were gated to the R-wave to exclude cardiac cycle-related changes in vessel diameter.

A resting scan was recorded over the course of 10 seconds to assess baseline vessel diameter. Increased forearm blood flow was induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the arterial segment being scanned) to a pressure of 250 to 300 mm Hg for 5 minutes, followed by release. A second scan was commenced 30 seconds before release of the cuff and continued for 90 seconds after deflation. After a period of 30 minutes to allow for vessel recovery, repeat resting images were obtained. Sublingual GTN spray (400 µg) was then administered to induce endothelium-independent vasodilatation, and a further 2-minute scan was performed 3 minutes later. The interexperiment coefficient of variation for flow-mediated vasodilatation assessment was 12.7%.

**Assessment of Exercise-Induced CPC Mobilization**

On a subsequent day, subjects underwent individualized exercise protocols involving a 15-minute period of rest, followed by 6 minutes of 20-watt cycling, and then 30 minutes at a workload equivalent to 80% of their individual lactate threshold. Cardiopulmonary variables were measured throughout as described. In addition, systolic and diastolic blood pressures were measured every 5 minutes by auscultation of the brachial artery, and capillary blood samples were taken every 10 minutes for measurement of lactate concentration (GM-7; Analox Instruments). Venous blood samples were taken immediately before and 20 minutes after exercise for enumeration of CPC and quantification of their mobilization (expressed as the percent increase in CPC numbers after exercise, relative to before exercise or, in other words, 100[CPC numbers after exercise/exercise CPC numbers before exercise] – 100) in response to subischemic exercise.

**NO Synthase Inhibition and CPC Mobilization in Response to Exercise**

In a group of 8 subjects, the protocol was repeated during the intravenous administration of the competitive NO synthase inhibitor L-NMMA (Clnalfa; Bachem GmbH); 0.9% sodium chloride solution (NaCl) was used as a control agent and norepinephrine was used as a second control agent with vasopressor action independent of NO synthase inhibition. An intravenous catheter was placed in the right antecubital fossa and connected to a syringe driver (NFuze N104; Daray) containing a 10-mL bolus of l-NMMA 3 mg/kg or 0.9% NaCl (for NaCl and norepinephrine experiments), which was delivered over the course of 5 minutes. Subsequently, an infusion of 0.9% NaCl, l-NMMA 3 mg/kg per hour, or 50 ng/kg per minute norepinephrine was commenced 15 minutes before exercise and continued until postexercise blood samples were collected. Experiments were separated by a minimum of 3 days and were conducted in a single blind manner.

**Circulating Progenitor Cell Enumeration**

Venous blood samples (4 mL) were stored in EDTA-coated tubes. Samples were mixed with equal volumes of phosphate-buffered saline, and then Ficoll PaquePLUS (GE Healthcare) density-gradient centrifugation was performed as per manufacturer’s instructions. Peripheral blood mononuclear cells were washed and suspended in phosphate-buffered saline at a concentration of 107 cells/mL; 100-µL aliquots of cell suspension were incubated with FcR Blocker (Miltenyi Biotech) to prevent nonspecific antibody binding. Appropriate volumes of the following antibodies, or their respective isotype controls, were then added: CD34-FITC (Miltenyi Biotech); CD133-APC (Miltenyi Biotech); and KDR-PE (R&D). In separate tubes, CD45-PE (Miltenyi Biotech), CD34-FITC (Miltenyi Biotech), or their corresponding isotype controls were added. CPC were enumerated using flow cytometry (BD FACSCalibur) using separate samples to measure CD34+/CD45–, CD34+/KDR+, and CD133+/CD45–/KDR+ subtypes. Isotype control specimens were used to define the threshold for antigen presence and to subtract nonspecific fluorescence. The cytometer was set to acquire 100,000 events within the lymphocyte gate, defined by typical light scatter properties (see Supplementary information available online at http://atvb.ahajournals.org).
Endothelial Microparticle Enumeration

Citrated blood samples were collected from the first 10 white European and 9 South Asian men studied. Samples were centrifuged at 11 000 g for 4 minutes to derive platelet-free plasma, which was further centrifuged at 20 000 g for 45 minutes to produce a microparticle pellet. This was then resuspended in 1 mL FACS buffer to produce an endothelial microparticle solution; 60-μL aliquots of endothelial microparticle solution were mixed with FcR blocker (Miltenyi Biotech) and appropriate concentrations of CD144 PE-conjugated antibody (R&D) or a corresponding isotype control. After labeling, samples were diluted to a total of 950 μL using FACS buffer and 50 μL counting bead suspension and were processed in the flow cytometer. FACS size calibration beads were used to gate analysis to submicron particles on the basis of laser forward-scatter properties. Absolute endothelial microparticle concentrations were derived using simultaneously collected counting bead data. 

Statistical Analysis

Initial pilot studies indicated a sample size of 15 subjects per group was necessary to demonstrate a 33% reduction in exercise-induced CPC mobilization in South Asian men (α=0.05; β=0.05). Based on combined data from the 2 ethnic groups studied, and assuming a 50% reduction in CPC mobilization during L-NMMA infusion, a sample size of 8 is required to detect a difference between samples (α=0.05; β=0.02). Continuous data are described as arithmetic mean and standard error of the mean. Comparison of continuous data from exercise studies uses Student unpaired and paired t tests when appropriate. Correlation between variables was assessed using linear or exponential regression as stated. Statistical significance was accepted at P<0.05; although when multiple comparisons are made, a Bonferroni correction is applied. All statistical analyses were performed using SPSS version 13.0 (SPSS).

Results

Subject characteristics are shown in Table 1. Groups were well-matched for age, body mass index, waist-to-hip ratio, blood pressure, fasting lipids, and glucose; however, the white European group was less insulin-resistant (homeostasis index of insulin resistance, 0.8±0.1 vs 1.3±0.2; P=0.047). Within the South Asian group, 13 defined ethnic background as Indian, 1 defined ethnic background as Pakistani, and 1 defined ethnic background as Sri Lankan.

Endothelial Damage and Conduit Endothelial Dysfunction

Endothelial dysfunction/damage was apparent in the South Asian sample as demonstrated by reduced brachial artery flow-mediated vasodilatation (5.8%±0.4% vs 7.9%±0.5%; P=0.002) and elevated CD144⁺ endothelial microparticles (281±41/μL vs 158±41/μL; P=0.03) compared to the white European group.

Exercise-Induced CPC Mobilization

Resting CPC numbers were reduced in the South Asian cohort for all 3 subsets of cells measured (Table 1; CD34⁺/KDR⁺, 0.030% vs 0.046%; P=0.05; CD133⁺/CD34⁺/KDR⁺, 0.008% vs 0.014%; P=0.026; and CD34⁺/CD45⁻, 0.011% vs 0.018%; P=0.044). As demonstrated in Figure 1 and Table 1, CPC mobilization in response to 30 minutes of exercise (at a steady state VO₂ of 84.5%±1.3% and 82.7%±2.1% lactate threshold in the South Asian and white European groups, respectively; P=0.49) occurred in South Asian and white European men. The magnitude of this increment was significantly lower in the South Asian men (CD34⁺/KDR⁺, 53.2%±6.9% vs 85.4%±5.1%; P=0.001; CD133⁺/CD34⁺/KDR⁺, 48.3%±8.8% vs 73.9%±9.1%; P=0.05; and CD34⁺/CD45⁻, 49.3%±7.9% vs 78.4%±5.7%; P=0.006).

Table 1. Cohort Characteristics

<table>
<thead>
<tr>
<th></th>
<th>White European</th>
<th>South Asian</th>
<th>P</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>28.2 (1.3)</td>
<td>30.0 (1.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.0 (0.7)</td>
<td>23.4 (0.7)</td>
<td>0.77</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.83 (0.01)</td>
<td>0.85 (0.02)</td>
<td>0.54</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>121 (2.1)</td>
<td>117 (2.7)</td>
<td>0.31</td>
</tr>
<tr>
<td>Lactate threshold, mL/kg/min</td>
<td>23.3 (1.4)</td>
<td>18.8 (0.7)</td>
<td>0.008</td>
</tr>
<tr>
<td>VO₂ max, mL/kg/min</td>
<td>48.2 (2.7)</td>
<td>38.0 (1.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.9 (0.2)</td>
<td>4.8 (0.1)</td>
<td>0.73</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.8 (0.1)</td>
<td>1.3 (0.2)</td>
<td>0.047</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.8 (0.2)</td>
<td>2.9 (0.1)</td>
<td>0.65</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.3 (0.1)</td>
<td>1.2 (0.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Total cholesterol-to-HDL cholesterol ratio</td>
<td>3.8 (0.4)</td>
<td>4.0 (0.2)</td>
<td>0.60</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.9 (0.5)</td>
<td>5.8 (0.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>GNT-induced vasodilatation, %</td>
<td>15.2 (8.8)</td>
<td>14.3 (6.6)</td>
<td>0.38</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>4.27 (0.16)</td>
<td>4.04 (0.1)</td>
<td>0.23</td>
</tr>
<tr>
<td>EMP, per μL plasma</td>
<td>157.9 (29.3)</td>
<td>281.3 (39.1)</td>
<td>0.03</td>
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<tr>
<td>CPC data before exercise</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CD34⁺/CD45⁻ CPC, % lymphocytes</td>
<td>0.018 (0.002)</td>
<td>0.011 (0.002)</td>
<td>0.044</td>
</tr>
<tr>
<td>CD34⁺/KDR⁺ CPC, % lymphocytes</td>
<td>0.046 (0.006)</td>
<td>0.030 (0.006)</td>
<td>0.05</td>
</tr>
<tr>
<td>CD133⁺/CD34⁺/KDR⁺ CPC, % lymphocytes</td>
<td>0.014 (0.002)</td>
<td>0.008 (0.001)</td>
<td>0.026</td>
</tr>
<tr>
<td>CPC data after exercise</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CD34⁺/CD45⁻ CPC, % lymphocytes</td>
<td>0.033 (0.005)</td>
<td>0.017 (0.004)</td>
<td>0.012</td>
</tr>
<tr>
<td>CD34⁺/KDR⁺ CPC, % lymphocytes</td>
<td>0.085 (0.012)</td>
<td>0.045 (0.008)</td>
<td>0.009</td>
</tr>
<tr>
<td>CD133⁺/CD34⁺/KDR⁺ CPC, % lymphocytes</td>
<td>0.027 (0.006)</td>
<td>0.011 (0.002)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Data presented as mean (standard error).

EMP indicates endothelial microparticles; FMD, flow-mediated vasodilatation; HDL, high-density lipoprotein; HOMA-IR, homeostasis index of insulin resistance; LDL, low-density lipoprotein.

Data collected from 15 subjects in each group.
Correlation Between Exercise-Induced CPC Mobilization and Flow-Mediated Vasodilatation

Within the entire study sample, significant correlation between flow-mediated vasodilatation and CPC mobilization (for all 3 subsets of CPC) was apparent (Figure 2). R values for CD34+/CD45−, CD34+/KDR+, and CD133+/CD34+/KDR+ cells were 0.61 (P<0.001), 0.41 (P=0.023), and 0.39 (P=0.035), respectively. Of note, 1 data point was removed from the analysis of correlation between CD133+/CD34+/KDR+ mobilization and flow-mediated vasodilatation in view of an anomalous decrease in cells during exercise (not witnessed during any other experiments) that was >2 standard deviations from the remaining data set.

NO Synthase Inhibition and Exercise-Induced CPC Mobilization

Figure 3 and Table 2 demonstrate CPC mobilization during infusion of NaCl, l-NMMA, and norepinephrine. For all 3 subsets of CPC analyzed, exercise-induced mobilization was significantly attenuated during l-NMMA infusion compared with NaCl (CD34+/KDR+, −3.3% vs 68.4%; P<0.001; CD133+/CD34+/KDR+, 0.7% vs 71.4%; P<0.001; and CD34+/CD45−, −30.5% vs 77.8%, P<0.001). In fact, for CD34+/KDR+ and CD133+/CD34+/KDR+ cells, mobilization was essentially absent, whereas for CD34+/CD45− analysis CPC mobilization was blunted below baseline. Importantly, a steady-state below the lactate threshold (84±7%) was achieved in all conditions. The relative exercise intensity (moderate) therefore was well-controlled between subjects and infusion conditions (Table 2). Exercise-induced CPC mobilization was unaffected by norepinephrine infusion (CD34+/KDR+, 78.9% vs 68.4%; P=0.47; CD133+/CD34+/KDR+, 62.6% vs 71.4%; P=0.72; and CD34+/CD45−, 88.1% vs 77.8%; P=0.74). During exercise, systolic and diastolic blood pressures were significantly increased, and
heart rate was significantly decreased during L-NMMA and norepinephrine infusion compared to control. The overall hemodynamic alterations induced by L-NMMA during exercise were reproduced during norepinephrine infusion, as intended.

Discussion

The present report presents a number of novel findings: (1) healthy South Asian men residents in the United Kingdom have lower CPC based on 3 different surface marker definitions, reduced NO bioavailability, and evidence of increased endothelial cell damage; (2) in response to moderate exercise, South Asian men have significant blunting of CPC mobilization; and (3) CPC mobilization in response to exercise is critically dependent on NO generation and correlates with the vasodilator response to forearm ischemia.

CPC Mobilization

Endothelial repair was originally thought to occur by migration and proliferation of neighboring endothelial cells. However, mature endothelial cells in vivo are terminally differentiated, with a low proliferative potential and limited capacity to regenerate damaged endothelium. Compelling evidence now supports the concept that in response to vascular injury, stem cells are mobilized from the bone marrow into the circulation, home to sites of endothelial cell damage, and contribute to vascular repair. Because there are currently a number of problems with delivery of cells as an approach to therapeutic vascular repair, understanding the

Table 2. Exercise Hemodynamic and Metabolic Data

<table>
<thead>
<tr>
<th></th>
<th>NaCl</th>
<th>L-NMMA</th>
<th>NE</th>
<th>P for NaCl vs L-NMMA</th>
<th>P for NaCl vs NE</th>
<th>P for L-NMMA vs NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>150 (4.6)</td>
<td>154 (5.0)</td>
<td>158 (5.9)</td>
<td>0.005</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71 (2.4)</td>
<td>77 (2.3)</td>
<td>79 (1.1)</td>
<td>0.015</td>
<td>0.016</td>
<td>0.334</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>123 (3.4)</td>
<td>114 (3.9)</td>
<td>116 (4.0)</td>
<td>0.016</td>
<td>&lt;0.001</td>
<td>0.647</td>
</tr>
<tr>
<td>Steady state VO2 as % lactate threshold</td>
<td>85 (2.4)</td>
<td>82 (2.4)</td>
<td>85 (1.9)</td>
<td>0.08</td>
<td>0.36</td>
<td>0.07</td>
</tr>
<tr>
<td>Capillary lactate, mmol/L</td>
<td>1.21 (0.18)</td>
<td>1.26 (0.27)</td>
<td>1.37 (0.26)</td>
<td>0.77</td>
<td>0.59</td>
<td>0.97</td>
</tr>
<tr>
<td>CPC data before exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+/CD45− CPC, % lymphocytes</td>
<td>0.014 (0.003)</td>
<td>0.016 (0.003)</td>
<td>0.009 (0.001)</td>
<td>0.68</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>CD34+/KDR+ CPC, % lymphocytes</td>
<td>0.035 (0.006)</td>
<td>0.035 (0.006)</td>
<td>0.03 (0.005)</td>
<td>0.93</td>
<td>0.48</td>
<td>0.55</td>
</tr>
<tr>
<td>CD133+/CD34+/KDR+ CPC, % lymphocytes</td>
<td>0.01 (0.001)</td>
<td>0.011 (0.001)</td>
<td>0.01 (0.002)</td>
<td>0.57</td>
<td>0.93</td>
<td>0.65</td>
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<tr>
<td>CPC data after exercise</td>
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<tr>
<td>CD34+/CD45− CPC, % lymphocytes</td>
<td>0.025 (0.006)</td>
<td>0.011 (0.002)</td>
<td>0.017 (0.003)</td>
<td>0.04</td>
<td>0.27</td>
<td>0.08</td>
</tr>
<tr>
<td>CD34+/KDR+ CPC, % lymphocytes</td>
<td>0.06 (0.01)</td>
<td>0.034 (0.006)</td>
<td>0.053 (0.006)</td>
<td>0.04</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>CD133+/CD34+/KDR+ CPC, % lymphocytes</td>
<td>0.017 (0.002)</td>
<td>0.011 (0.001)</td>
<td>0.016 (0.003)</td>
<td>0.02</td>
<td>0.64</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Data presented as mean (standard error).
BP indicates blood pressure; NE, norepinephrine.
Blood pressure and heart rate data represent mean value over a 30-minute period of exercise. Capillary lactate data are after 30 minutes of exercise. Data collected from 8 subjects.
mechanisms by which CPC are mobilized in humans is of particular importance.

Role of NO in CPC Mobilization
Experimental models have shown that NO is critical for CPC mobilization in response to physiological stimuli, such as exercise, and pathophysiological cues, such as ischemia. The role of NO in the mobilization of CPC in healthy humans and those at high risk for atherosclerosis has been unclear. In the present study, we have shown that CPC mobilization correlates closely with brachial artery flow-mediated vasodilatation, a well-established measurement of peripheral vascular NO bioavailability. Moreover, we have shown, for the first time to our knowledge, in humans that inhibiting NO production using the nonselective NO synthase inhibitor L-NMMA leads to complete blockade of CPC mobilization in response to moderate-intensity exercise. These data firmly establish a pivotal role for NO in CPC mobilization in humans. Recent data from Goto et al. provided insight into how aerobic cycling exercise might augment CPC mobilization in an NO-dependent manner. During moderate-intensity cycle exercise they demonstrated an NO-dependent increase in forearm blood flow, presumably via enhanced shear stress induced by increased cardiac output. Hence, it is possible that such exercise may induce enhanced shear (and therefore NO) within the bone marrow vasculature or stimulate release of circulating humoral factors, resulting in enhanced progenitor cell release from the stem cell niche. Of course, we cannot be certain of the origin of CPC measured during these studies; therefore, other possibilities such as origins not of the bone marrow remain possible. Ethical and technical issues constrain the ability to perform studies tracking the origin and destination of such cells. Data from murine studies suggest that modulation of bone marrow NO, with subsequent augmentation of matrix metalloproteinase-9 activity, and soluble Kit ligand release are crucial for mobilization of CPC into the circulation from their quiescent extravascular bone marrow niche. It is tempting to speculate that such mechanisms are relevant in human physiology, although our work cannot provide confirmation of this.

South Asian Ethnicity and CPC Biology
Pathological studies have demonstrated a defined series of changes in the vessel wall during atherogenesis. There is now compelling evidence that very early in the atherogenic process, before the onset of morphological changes, a subtle phenotypic change in the endothelium occurs. Arguably, the most crucial feature of this process is a decline in the bioavailability of the antiatherosclerotic signaling molecule NO. The seminal article by Ross proposing the “response to injury” hypothesis established endothelial damage/dysfunction as a key stage in the complex portfolio of pathological steps in the development of occlusive arterial disease. Consistent with this paradigm, longitudinal studies have shown that endothelial cell dysfunction is a predictor of the development of coronary artery atherosclerosis and coronary events. Our own work and that of other groups has demonstrated endothelial dysfunction in South Asian men. In the present study, we have confirmed these findings and also show for the first time to our knowledge that healthy South Asian men residents in the United Kingdom have increased circulating endothelial cell-derived microparticles, which are a marker of accelerated endothelial cell loss. Despite this accelerated endothelial damage/dysfunction, we have shown that South Asian men have a reduction of a range of CPC subtypes. Importantly, the CD34+/KDR+ fraction we measured has previously been demonstrated to prospectively predict major cardiovascular events. Furthermore, recent work has demonstrated that within the CD34+/CD45+ fraction reside the in vitro-derived, late-outgrowth endothelial progenitor cells, a cell population that is speculated to participate in in vivo endothelial repair.

The mechanisms underlying this apparent reduction in endogenous repair capacity are unclear. However, whereas it may be an oversimplification, CPC bioavailability is likely to be a balance between release of progenitors from the bone marrow stem cell niche and their survival or rate of utilization after release. Mobilization is a critical first step in CPC-mediated repair, and we have clearly demonstrated a reduced ability to mobilize CPC in South Asian men in response to a carefully controlled exercise stimulus. It is also tempting to speculate that this is attributable to reduced NO bioavailability because we have shown that mobilization is critically NO-dependent.

Conclusions
In summary, we have demonstrated that exercise-induced CPC mobilization is reduced in healthy South Asian men resident in the United Kingdom. We have also shown that mobilization correlates with in vivo measurements of NO bioavailability, and that CPC mobilization can be blocked by NO synthase inhibition. Therefore, reduced exercise-mediated CPC mobilization in South Asian men is likely to be secondary to the reduced NO bioavailability also noted during our studies; therefore, interventions to enhance CPC mobilization by augmenting NO bioavailability warrant further study.

Sources of Funding
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References


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Supplement Material

Supplement Figure I

Supplement Figure I: Flow cytometric gating strategy for EPC enumeration.
Panel A shows a standard FSC/SSC scatter-plot with the lymphocyte region contained in gate P1. Panel C demonstrates CD34 by CD45 analysis (Corresponding isotype control experiments in panels B); Cells in Q4 represent CD45-/CD34+ progenitors. Panel E demonstrates CD34 by KDR analysis (Corresponding isotype control experiments in panels D); Cells in Q2 represent CD34+/KDR+ progenitors. Panel F represents a CD133 histogram of CD34+/KDR+ progenitors (from Q2 of Panel E); Cells in gate P2 are CD133+CD34+/KDR+ progenitors.