Vascular Dysfunction and Reduced Circulating Endothelial Progenitor Cells in Young Healthy UK South Asian Men

Cliona Murphy, Gajen S. Kanaganayagam, Benyu Jiang, Philip J. Chowienczyk, Rainer Zbinden, Mrinal Saha, Salman Rahman, Ajay M. Shah, Michael S. Marber, Mark T. Kearney

Objectives—The objective of this study was to examine determinants of excess coronary artery disease risk in UK South Asians, more prevalent in this population than UK Caucasians, by examining differences in risk factors, vascular function, and endothelial progenitor cells (EPCs).

Methods and Results—24 South Asian and 25 Caucasian healthy age-matched nonsmoking men were studied. Vascular function was assessed by flow-mediated and GTN brachial artery dilatation and blood flow responses to infusion of ACh, SNP, and L-NMMA. EPC number and function were measured by flow cytometry (CD34, CD133, and KDR positive cells), and CFU/migration assays. Traditional risk factors and anthropometric measurements were similar in the groups. South Asians had higher fasting insulin levels (6.01 versus 3.62 μU/mL; \( P = 0.02 \)). South Asians had lower FMD (6.9 versus 8.5%; \( P = 0.003 \)), L-NMMA response (0.8 versus 1.3 mL/min/100 mL; \( P = 0.03 \)), mean SNP response (9.5±0.6 versus 11.6±0.6; \( P = 0.02 \)), EPC number (0.046±0.005% versus 0.085±0.009%; \( P = < 0.001 \)), and CFU ability (CFU 4.29±1.57 versus 18.86±4.00; \( P = 0.005 \)). EPC number was the strongest predictor of FMD. Ethnicity was the strongest predictor of EPC number.

Conclusions—Healthy South Asian men are more insulin resistant, and demonstrate endothelial dysfunction and reduced EPC number and function compared with Caucasians. These abnormalities may contribute to their increased CAD risk.

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Key Words: endothelium ■ vascular dysfunction ■ endothelial progenitor cells ■ nitric oxide ■ insulin resistance

It is well established that migrant South Asians are at higher risk of coronary artery disease (CAD) and its complications than White Caucasians.\(^1\)\(^2\) Data from the 1991 England and Wales Census showed that CAD mortality rates were 40% to 50% higher in South Asians than in the total population.\(^3\) The SHARE Study demonstrated that the prevalence of CAD in South Asians is over twice that of White Europeans.\(^4\) Conventional risk factors, although important in predicting CAD risk in both groups, do not explain the higher risk in South Asians.\(^5\) Insulin resistance and/or visceral adiposity may contribute some of the excess risk.\(^6\) A UK study demonstrated that South Asian children (aged 8 to 11 years) already have abnormalities of glucose homeostasis compared with age-matched White children.\(^7\) Insulin resistant individuals have a significantly increased risk of cardiovascular disease. The underlying mechanisms are incompletely understood but are likely to be multifactorial.\(^8\) Endothelial dysfunction may play a key role early in the process by initiating and potentiating the development of atherosclerosis and, indeed, several studies have shown evidence of endothelial dysfunction in insulin resistant states.\(^9\)\(^10\) A hallmark of endothelial dysfunction is a reduction in the bioavailability of endothelium-derived nitric oxide (NO), a molecule with a variety of antiatherogenic properties.\(^11\) Numerous studies have demonstrated impairment of the NO-mediated vasodilatory response in the peripheral and coronary circulations of patients with cardiac risk factors or established atherosclerosis.\(^12\)\(^13\) The degree of impairment is related to the severity and extent of CAD.\(^14\) Relevant to the pathogenesis of such dysfunctional endothelium, the concept of repair of damaged/dysfunctional endothelial cells by endothelial progenitor cells (EPCs) has recently emerged. Bone marrow–derived EPCs released into the circulation are thought to home to areas of endothelial injury and replace damaged endothelium.\(^15\)\(^16\) In addition, EPCs have been shown to participate in neovascularization in numerous animal models.\(^17\)\(^18\) The selective recruitment of EPCs to areas of injury and neovascularization is thought to be mediated, in part, by local production of the chemokine stromal cell derived factor-1 (SDF-1) and corresponding EPC expression of the chemokine receptor CXCR4.\(^19\) Previous studies have shown that numbers of circulating EPCs are diminished in patients with established
CAD, are predictive of future cardiovascular events, and positively correlate with measures of endothelial function.20,21 This study examined whether or not the higher CAD risk in South Asian men might be explained by reduced endothelial function and numbers or function of circulating EPCs.

Methods

Study Subjects

49 healthy men living in the UK were studied. 24 South Asian and 25 White Caucasian nonsmoking, age-matched (20 to 40 years) men, without traditional risk factors for CAD, were recruited for the study by local and college advertisement. South Asian ethnicity was defined as first or second generation migrants, with both parents of native South Asian origin (from the countries of India, Bangladesh, Sri Lanka, or Pakistan). Subjects gave informed consent and protocols were approved by the University ethics committee in accordance with the Helsinki Declaration II. During the study, subjects were not taking prescribed medication or vitamin/herbal supplements. Subjects were studied after an overnight fast in a temperature-controlled room at 22°C and were asked to refrain from alcohol and caffeine-containing beverages for 24 hours before the study.

Exercise

Subjects were asked to complete a one week questionnaire detailing exercise taken (formal eg, attending the gym and non-formal eg, walking to work) during that week.

Anthropometric and Blood Pressure Measurements

Height and weight were recorded without shoes in light clothing. Waist circumference was measured at the level of the umbilicus and hip circumference was measured at the level of the femoral trochanter. Blood pressure, taken after 15 minutes rest, was recorded as the mean of 3 supine readings using an Omron 705 oscillometric monitor. Blood pressure, taken after 15 minutes rest, was recorded as the mean of 3 supine readings using an Omron 705 oscillometric monitor (Omron).

Biochemical Assays

Fasting venous blood was collected for measurement of levels of insulin, glucose, total and high-density lipoprotein cholesterol, triglycerides, and high sensitivity C-reactive protein. Samples were immediately centrifuged at 4°C (15 minutes, 4000 rpm) and serum and plasma were stored at −80°C for later analysis. HsCRP levels (high sensitivity C-reactive protein) were measured in mg/L using a high sensitivity assay (Dade-Behring). Plasma insulin levels were measured by ELISA assay (DPC Immulite). As a measure of insulin resistance, the homeostasis model assessment index (HOMA-IR) was calculated as fasting plasma insulin (uU/mL) × fasting glucose (mmol/L)/22.5. Levels of SDF-1 were measured by ELISA (Tebu-Bio).

Assessment of Conduit Vessel Endothelial Function: Flow Mediated Dilatation

Noninvasive assessment of brachial artery vascular reactivity was performed using high resolution B-mode ultrasound (Accuson 12XP/10 system) with a 7-MHz linear-array transducer, as previously described.12 The brachial artery was scanned longitudinally with the Helsinki Declaration II. During the study, subjects were not taking prescribed medication or vitamin/herbal supplements. Subjects were studied after an overnight fast in a temperature-controlled room at 22°C and were asked to refrain from alcohol and caffeine-containing beverages for 24 hours before the study.

Assessment of Conduit Vessel Endothelial Function: Flow Mediated Dilatation

Noninvasive assessment of brachial artery vascular reactivity was performed using high resolution B-mode ultrasound (Accuson 12XP/10 system) with a 7-MHz linear-array transducer, as previously described.12 The brachial artery was scanned longitudinally above the antecubital fossa, with an inflatable cuff around the forearm. The transducer was held in position using a purpose-designed clamp after optimization of image quality. A baseline image was recorded and then the cuff was inflated to at least 50 mm Hg above systolic blood pressure for 5 minutes. Images were continuously recorded for 2 minutes after cuff deflation during reactive hyperemia. Flow mediated dilation (FMD) was taken as the maximal change in dilatation from baseline during the period 60 to 90 seconds after cuff deflation and was expressed as a percentage of the baseline diameter. The percentage dilatation in response to GTN (Mayne Pharma), 0.4 mg sublingually was used as a measure of endothelium-independent dilatation. All measurements were performed using an automated edge detection system (Brachial analysis system version 3.2, Medical Imaging Applications).

Assessment of Resistance Vessel Endothelial Function: Forearm Blood Flow

Forearm blood flow (FBF) responses in both arms were measured by a Hokanson model EC6 venous occlusion strain gauge plethysmograph apparatus which was electrically calibrated as previously described.22 The brachial artery of the nondominant arm was cannulated with a 27-gauge needle under local anesthesia (0.1% lignocaine) and used for drug infusions. All drugs were dissolved in 0.9% saline. After baseline measurements during infusion of saline alone, FBF was measured during sequential intrabrachial infusions of cumulative doses of ACh (Miochol - Novartis), 15 and 30 μg/min each for 5 minutes; two cumulative doses of SNP (Mayne Pharma), 3.0 and 10.0 μg/min each for 5 minutes; and a single dose of L-NMMA (Clinalfa), 8 μmol/min, for 7.5 minutes. Between administrations of drugs, normal saline was infused for at least 10 minutes or until blood flow returned to baseline rates. A wrist cuff was inflated to suprasystolic pressures before each recording, to exclude the hand circulation. FBF was measured during the last 2.5 minutes of each infusion period and recorded as the mean of 5 venous occlusion readings. FBF was expressed as ml/min/100 mL forearm volume. Mean responses to Ach and SNP were calculated as the average of the FBF at each infused dose.

Circulating EPC Number

A 2-mL blood sample was used for enumeration of circulating endothelial progenitor cells. Mononuclear cells were separated from other components of peripheral blood by centrifugation on density gradient media (Histopaque 1077, Sigma). EPCs were identified by flow cytometry as cells coexpressing 3 characteristic antigens, the hematopoietic progenitor cell marker CD34, the immature hematopoietic progenitor cell marker CD133, and the endothelial cell receptor VEGFR2, as previously described.23 To block nonspecific binding of antibodies, cells were incubated with an Fc receptor blocker (Miltenyi Biotech). The CD133 antigen was stained by incubation with a primary CD133 antibody (Miltenyi Biotech) followed by a secondary fluorescein isothiocyanate (FITC) conjugate (Dako). CD34 and VEGFR2 antigens were stained by incubation with preconjugated antibodies to PerCP and PE, respectively (R&D Systems). Cells expressing the CXCR4 receptor were identified by staining with a FITC-preconjugated CXCR4 antibody (R&D Systems). Control isotype- and species-matched antibodies were used in each analysis. Stained cells were resuspended and analyzed by 3-color flow cytometry (FACScan - Becton Dickinson). The cytometer was set to acquire 200 000 events, and analyses were performed within the lymphocyte gate, in accordance with a technique used by other investigators.21 Analyses were performed in a blinded fashion using a Win MDI 2.8 software program. Antigen expression/staining was recorded as the mean fluorescence intensity. Single antigen staining values were calculated by histogram subtraction of control from active fluorescence values. Dual staining values were quantified similarly using a dot plot, and triple staining was quantified using Boolean logic applied to gates of positively stained cells from two dot plots. The coefficient of variation was 30% on 5 synchronous samples using this method of triple antigen staining EPC enumeration.

EPC Function

Colony-Forming Unit Assay and NO Production

EPC functional studies were undertaken in a subset of the subjects. 10 subjects from each study group were invited back for analysis of cell properties. Of these, 11 subjects, 6 South Asian and 5 Caucasian, were restudied. Isolated peripheral blood mononuclear cells from a 25-ml blood sample were cultured on fibronectin-coated (Sigma, 10 μg/ml) 12-well plates (BD). Cells were grown in EBM-2 with SingleQuots (Clonetics), containing fetal bovine serum, hydrocorti-
TABLE 1. Anthropometric, Clinical, and Biochemical Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Asian Subjects (n=24)</th>
<th>Caucasian Subjects (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>25.6±5.4</td>
<td>26.3±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.6±7.9</td>
<td>79.1±10.5</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.7±2.6</td>
<td>24.3±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86±0.05</td>
<td>0.85±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>115±7.7</td>
<td>119±6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71±6.3</td>
<td>69±7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>86±5.8</td>
<td>86±6.9</td>
<td>NS</td>
</tr>
<tr>
<td>TChol, mmol/L</td>
<td>4.09±0.16</td>
<td>4.13±0.13</td>
<td>NS</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.18±0.05</td>
<td>1.18±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.89±0.11</td>
<td>0.98±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.02±0.19</td>
<td>1.13±0.31</td>
<td>NS*</td>
</tr>
<tr>
<td>Fasting</td>
<td>4.82±0.15</td>
<td>4.88±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.01±0.72</td>
<td>3.62±0.40</td>
<td>0.02*</td>
</tr>
<tr>
<td>Insulin, uU/ml</td>
<td>1.31±0.16</td>
<td>0.77±0.09</td>
<td>0.01*</td>
</tr>
<tr>
<td>HOMA-IR Index</td>
<td>320.2±128.8</td>
<td>162.8±45.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Subject characteristics expressed as means±SD (data above dotted line). biochemical data expressed as means±SEM. *Nonparametric analysis.

TABLE 2. Conduit Vessel Function

<table>
<thead>
<tr>
<th></th>
<th>Asian Subjects (n=24)</th>
<th>Caucasian Subjects (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline brachial artery diameter, mm</td>
<td>3.8±0.1</td>
<td>3.9±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>GTN dilatation, %</td>
<td>10.7±0.8</td>
<td>11.5±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>FMD, %</td>
<td>6.9±0.3</td>
<td>8.5±0.4</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM.

TABLE 3. Resistance Vessel Function

<table>
<thead>
<tr>
<th></th>
<th>Asian Subjects (n=24)</th>
<th>Caucasian Subjects (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline saline 1 mL/min</td>
<td>2.0±0.2</td>
<td>2.4±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>L-NMMA, ΔFBF</td>
<td>0.8±0.1</td>
<td>1.3±0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>ACh 15 μg/min</td>
<td>7.2±0.7</td>
<td>9.6±0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>ACh 30 μg/min</td>
<td>9.4±1.0</td>
<td>10.9±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>ACh mean</td>
<td>8.1±0.8</td>
<td>10.3±0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>SNP 3 μg/min</td>
<td>8.2±0.5</td>
<td>9.4±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>SNP 10 μg/min</td>
<td>11.0±0.7</td>
<td>13.8±0.9</td>
<td>0.02</td>
</tr>
<tr>
<td>SNP mean</td>
<td>9.5±0.6</td>
<td>11.6±0.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Statistical Analysis

Data were analyzed using the SPSS PC statistical package (version 11.0, SPSS Inc). Subject characteristics are reported as means±SD and all other result as means±SEM. For normally distributed data, the unpaired Student t test was used to make comparisons between groups. The Mann–Whitney test was used to compare nonnormally distributed data (insulin, HOMA-IR, hsCRP, EPCs). P<0.05 was taken as indicating statistical significance, and all tests were 2-tailed. Univariate and multiple linear regression analyses were performed to examine associations between risk factors and measured FMD and EPCs.

Results

Anthropometric, Biochemical, and Exercise Characteristics

South Asian and Caucasian men had similar body mass index (BMI), waist-hip ratio (WHR), blood pressure, fasting lipid and glucose profiles, hsCRP, and SDF-1. South Asian men had significantly higher fasting insulin levels and HOMA insulin resistance scores than Caucasian men (Table 1). South Asian men tended to undertake less hours of formal exercise per week than Caucasians, but no subjects were sedentary.

Vascular Function

Baseline brachial artery diameter and GTN-mediated dilatation were similar in the 2 groups. South Asian men had significantly lower FMD than Caucasians (Table 2). In resistance vessels there was no difference in resting FBF. South Asian men had significantly lower FBF responses to both L-NMMA and SNP. South Asian subjects also had lower FBF responses to Ach, but the differences did not meet significance (Table 3).

Endothelial Progenitor Cells

Circulating EPC Numbers

The number of circulating EPCs was significantly lower in South Asian than in Caucasian men. Numbers of the larger group of CD133/CD34 hematopoietic stem cells were also lower. No difference was seen in circulating levels of SDF-1 (Table 1) or in progenitor cells expressing the SDF-1 receptor (CD34+ CXCR4+).

sone, human FGF, VEGF, R3-IGF, ascorbic acid, hEGF, heparin, and GA-1000, and supplemented with 20% fetal calf serum in an incubator at 37°C with 5% CO₂. Cells were plated for 2 days, and nonadherent cells were washed away at this time. Culture media was changed twice per week and colonies were counted on day 14 of culture.

Colony-forming units were counted per well using an eyepiece graticule on a light microscope (Nikon) and translated to number per cm². NO was indirectly measured in cell culture supernatant at day 14 of culture (after a change of culture media at day 12) using a plasma nitrite assay (Parameter Total NO/Nitrite/Nitrate assay; R&D).

Cell Migration Assay

Cells were washed with PBS (Sigma) on day 14 and incubated with 5 μg/mL Calcein (Molecular Probes) and 2% charcoal treated FBS for 2 hours. Cells were detached using 0.5 mmol EDTA/0.5% trypsin (Gibco) and washed in 3.5 mg/mL BSA in DMEM (PAA laboratories). Cells were placed in the upper chamber of a modified Boyden chamber (1 um BD Falcon HTS FluoroBlok were used) and precoated with fibronectin 10 μg/mL (Sigma) for 24 hours and loaded into a 24-well BD Falcon plates. 1 mL of 3.5 mg/mL BSA in DMEM was added into the lower chamber for control samples, and supplemented with 50 ng/mL SDF-1 (R&D) in test samples. Migration was measured as fluorescence intensity using a bottom plate reading fluorometer (Victor). All experiments were performed in triplicate.
**EPC Functional Characteristics**

The number of EPC colony forming units was significantly lower in South Asians compared with Caucasians.

Migration of cells toward SDF-1, measured in 3 South Asians (attributable to bacterial contamination in 1 sample and insufficient cell growth in 2 samples), and 4 Caucasians (attributable to bacterial contamination in 1 sample) showed a nonsignificant trend toward being greater in the Caucasian group.

The nitrite concentration within the cell culture supernatant was not significantly different between the two groups.

**Regression Analysis**

On multivariate regression analysis of vascular function, as assessed by flow mediated dilatation, against key variables (age, MAP, TChol, HOMA-IR, WHR, ethnicity, EPCs), the strongest predictor of FMD was EPC number ($B=0.43$, $t=2.46$, $P=0.02$; ANOVA $P=0.03$; Table 5). On multivariate regression of EPC number against the same variables, ethnicity was the strongest predictor of EPC count ($B=0.65$, $t=3.86$, $P=0.001$; ANOVA $P=0.03$; Table 6).

**Discussion**

Insulin resistance in South Asians has been previously attributed to relative visceral adiposity$^{6,24}$ and has been noted in association with heightened systemic inflammation as shown by higher concentrations of CRP than those seen in Caucasian subjects.$^{25,26}$ In the present study, subjects had similar gross measurements of adiposity and levels of CRP, suggesting that insulin resistance in young South Asian men may be independent of visceral adiposity and associated inflammation. The higher plasma insulin level in South Asians reflects a relative hyperinsulinemia which has been shown to be an independent risk factor for the development of CAD,$^{27}$ an effect thought to be mediated via an imbalance between the detrimental and favorable effects of insulin on the vasculature. Indeed, endothelial dysfunction is an integral part of the syndrome of insulin resistance, evident before the onset of hyperglycemia.$^{9}$ The HOMA-IR scores in this study are not within the clinical range of insulin resistance where treatment might be instigated. However, a higher HOMA-IR score, as was found in the South Asian subjects, has been shown to be associated with a higher risk of developing later diabetes, with epidemiological studies in UK South Asians confirming this.$^{5,6}$ Several studies have shown that insulin has a vasodilatory action mediated by NO production.$^{28,29}$ It is, therefore, possible that the endothelial dysfunction observed in South Asians may in part be a result of resistance to insulin-mediated eNOS activation.

FMD provides a technique to assess the integrity of the shear stress–mediated pathway of NO production. FMD of the brachial artery is closely correlated with coronary vaso-motor responses.$^{30}$ A reduction in FMD is thought to represent an early functional disturbance in the development of CAD. Consonant with this, FMD has been shown to be of prognostic value in several studies.$^{12,14}$ The healthy young South Asian men in this study demonstrated significantly lower brachial artery FMD, indicative of reduced NO bio-availability. This finding of endothelial dysfunction in South Asians is in line with that of a previous study of older UK South Asian men. In the latter study, however, South Asian subjects were significantly more viscerally obese than corresponding Caucasian subjects.$^{31}$

L-NMMA is an endogenous eNOS inhibitor. The significantly lower L-NMMA response in South Asian men provides evidence of reduced basal NO production. In line with

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**TABLE 4. Progenitor Cells**

<table>
<thead>
<tr>
<th>Percentage of Cells in the Lymphocyte Gate</th>
<th>Asian Subjects (n=24)</th>
<th>Caucasian Subjects (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPCs (CD34$^+$ CD133$^+$ VEGFR2$^+$)</td>
<td>0.046±0.005</td>
<td>0.085±0.009</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total hematopoetic stem cells (CD133$^+$ CD34$^+$)</td>
<td>0.078±0.009</td>
<td>0.117±0.010</td>
<td>0.008</td>
</tr>
<tr>
<td>Homing progenitor cells (CD 34$^+$ CXCR4$^+$)</td>
<td>0.068±0.026</td>
<td>0.091±0.011</td>
<td>NS</td>
</tr>
<tr>
<td>CFUs, per cm$^2$</td>
<td>4.29±1.57</td>
<td>18.86±4.00</td>
<td>0.005</td>
</tr>
<tr>
<td>NO release, umol/L</td>
<td>20.07±1.36</td>
<td>23.81±3.41</td>
<td>NS</td>
</tr>
<tr>
<td>Migration to SDF-1, % relative to control</td>
<td>3.03±3.03</td>
<td>19.23±6.06</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM. *Nonparametric analysis.

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**TABLE 5. Multivariate Analysis of FMD against Cardiovascular Risk Factors, Ethnicity and EPCs**

<table>
<thead>
<tr>
<th>B</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.228</td>
<td>1.50</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>−0.177</td>
<td>−1.12</td>
</tr>
<tr>
<td>TChol</td>
<td>0.127</td>
<td>0.84</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>−0.21</td>
<td>−1.17</td>
</tr>
<tr>
<td>WHR</td>
<td>−0.051</td>
<td>−0.31</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.116</td>
<td>0.58</td>
</tr>
<tr>
<td>EPCs</td>
<td>0.433</td>
<td>2.46</td>
</tr>
<tr>
<td>Significance, ANOVA</td>
<td></td>
<td>0.03</td>
</tr>
</tbody>
</table>
the FMD and L-NMMA responses, the vasodilatory response to acetylcholine was lower in South Asian subjects supporting the hypothesis that principally NO-mediated vasodilatory pathways are selectively impaired in young South Asian men. The ACh response, however, surprisingly did not meet significance, one explanation being that the sample size was not large enough to detect a significant difference, especially as the relative contribution of ACh-mediated NO production is lower in the resistance vasculature than conduit vessels. Vasodilatation in response to shear stress is almost exclusively NO-mediated and L-NMMA-induced vasoconstriction reflects NO production in response to isometric tension, whereas ACh stimulates vasodilatation by several pathways, some of which, such as EDHF mediated, are NO-independent. Therefore, another explanation for the relative preservation of the ACh response is that it may represent upregulation of ACh-stimulated EDHF vasodilatation in the context of decreased NO bioavailability. The data are consistent with our previous findings in murine models of insulin resistance demonstrating selective blunting of insulin-mediated (Ca^{2+}-independent) NO production with relatively preserved acetylcholine responses. The observation of reduced blood flow in response to the NO donor SNP in South Asian men may be the result of NO scavenging by free radicals or may reflect a reduction in vascular smooth muscle sensitivity to nitric oxide. No difference in the brachial artery response to GTN was seen, although it may be that the NO donor response in resistance vessels is affected earlier than in conduit arteries.

The lower number of circulating EPCs in South Asian men may have implications for endothelial health and homeostasis. A significant reduction in the number of EPCs would be expected to result in a reduced capacity for endothelial repair. Circulating EPC number is thought to be governed by various factors. Acute organ ischemia such as myocardial infarction provides a strong chemokine signal, mobilizing progenitors from the bone marrow into the circulation, whereas the chronic vascular injury, evident in conditions predisposing subjects to CAD such as diabetes, is associated with reduced circulating EPC numbers. Although chronic reductions may reflect known predisposing traditional cardiovascular risk factors, recent studies have also shown that circulating EPC number predicts the occurrence of cardiovascular events and death from cardiovascular causes independent of these factors. This supports the possibility that reduced EPC number, which in this study predicted FMD independently of insulin resistance, may be a primary abnormality in South Asians. Indeed ethnicity independently predicted circulating EPC count.

In addition to quantitative differences in EPCs, there was evidence of lower EPC functional capacity in the South Asian group in the form of proliferative capability and migratory response. In this study the cultured cells adherent at 14 days can be termed “early outgrowth” cells as described by others. The proliferative colony-forming ability of these cells was significantly lower in South Asians. In addition, although the migration study numbers were small, the migratory response of these cells toward SDF-1 did appear to be lower in the South Asian subjects, a possible indicator of both diminished intracellular signaling following activation of the SDF-1–CXCR4 axis and of intrinsic migratory capacity. These findings are important given that an impaired functional capacity of EPCs is a phenomenon seen in patients with established cardiovascular disease.

The role of the SDF-1–CXCR4 axis in EPC mobilization was also examined in the study. There was a nonsignificant trend toward an increased concentration of SDF-1 in South Asian subjects, indicating that certainly the SDF-1 mobilizing signal is not diminished. This result and the finding of a similar proportion of progenitors expressing the corresponding receptor (CXCR4) may be expected to reflect similar migratory responses. However, as indicated above, the cell culture studies demonstrated a nonsignificant trend toward reduced migratory response of cells from the South Asian group toward SDF-1. This is in keeping with the CFU assay, which incorporates a measure of cell function. The tendency to higher levels of SDF-1 in South Asians may possibly be explained by a homeostatic attempt at upregulation (albeit an unsuccessful one) to encourage further mobilization of the cells. Support for this concept is seen in the work of Heiss et al who noted higher levels of VEGF, with similar numbers of, but poorly functioning EPCs in older subjects.

A number of limitations of the present study should be discussed. As this was an observational and cross-sectional study, causality cannot be examined. Although we demonstrated impairment of the ability of EPCs from Asians in the colony forming unit assay, further mechanistic studies underlying the reduction in number and function of EPCs from young Asian men are required. Moreover, a larger sample should be studied to assess EPC migration as while our data showed a strong tendency the difference between ethnic...
groups this did not reach conventional levels of statistical significance. One should also exercise caution in interpretation of measurements of nitrite/nitrates, as a component of this could be attributable to iNOS - as well as eNOS-derived NO as we have recently demonstrated.\(^{47}\) The findings of the present study can only be applied to healthy males, and future studies will be needed to examine whether ethnic differences are demonstrable in female subjects and in those with overt cardiovascular risk factors. In addition it is not clear to what degree the observed metabolic and vascular abnormalities in South Asian migrants are genetic in origin or whether environmental factors such as adoption of a Westernized lifestyle and ethnic differences in diet and exercise habits might contribute to expression of the abnormalities. Detailed exercise and diet studies in migrant South Asians versus Caucasians are lacking in the literature, possibly because of the difficulty in measurement of each in practical terms, but this study did show that formal exercise might differ between these groups and larger more detailed studies are needed in this area. With interpretation of the results it should be noted that various EPC isolation and quantification techniques are described in the literature and as yet there is no standardized approach. In addition there is some ambiguity regarding precisely which antigens expressed on stem cells identify those capable of developing into endothelial cells, an area of ongoing research.

Despite the limitations noted above, the present study demonstrates a number of important findings. Healthy young South Asian men, compared with corresponding Caucasians of similar age and body habitus, are more insulin-resistant, have endothelial dysfunction evident in conduit and resistance vasculature, and have lower circulating numbers of EPCs and measures of EPC functionality. These findings may together contribute to the increased CAD risk in South Asians. Future interventional studies will be important in defining a possible mechanistic link between the findings and may improve our understanding of the pathophysiological processes leading to higher rates of CAD in migrant South Asian populations.

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Disclosures

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