Endothelium and Nitric Oxide

Asymmetric Dimethylarginine and Reduced Nitric Oxide Bioavailability in Young Black African Men

Narbeh Melikian, Stephen B. Wheatcroft, Okechukwu S. Ogah, Cliona Murphy, Phillip J. Chowienczyk, Anthony S. Wierzbicki, Thomas A.B. Sanders, Benyu Jiang, Edward R. Duncan, Ajay M. Shah, Mark T. Kearney

Abstract—Black Africans have a higher incidence of cardiovascular disease than white Europeans. We explored potential mechanisms of this excess risk by assessing endothelial function, inflammatory status (C-reactive protein), oxidative stress (isoprostane-F2α), and plasma asymmetrical dimethyl arginine (ADMA; an endogenous competitive inhibitor of NO synthase) in each ethnic group. Thirty healthy black Africans and 28 well-matched white European male subjects were studied (mean age±SE: 32.2±0.9 and 29.2±1.2 years, respectively; \( P=0.07 \)). High-resolution ultrasound was used to assess vascular function in the brachial artery by measuring flow-mediated dilatation ([percentage of change]; endothelium-dependent function) and glyceryltrinitrate dilatation ([percentage of change]; endothelium-independent function). Blood pressure, fasting lipids, glucose, and estimated glomerular filtration rate levels were similar in both groups. There was no difference in C-reactive protein (black Africans: 0.8±0.1 mg/L; white Europeans: 0.6±0.1 mg/L; \( P=0.22 \)), isoprostane-F2α (black Africans: 42.9±1.5 pg/mL; white Europeans: 39.2±1.5 pg/mL; \( P=0.23 \)), and leptin (black Africans: 64.1±10.2 ng/mL; white Europeans: 47.8±9.8 ng/mL; \( P=0.37 \)) levels between the 2 ethnic groups. However, compared with white Europeans, plasma ADMA levels were significantly higher in black Africans (0.34±0.02 μmol/L and 0.25±0.03 μmol/L; \( P=0.03 \)). There was no difference in the percentage of glycerylinitrate dilatation (\( P=0.7 \)), but the percentage of flow-mediated dilatation was significantly lower in black Africans (black Africans: 5.2±0.3; white Europeans: 6.3±0.4; \( P=0.02 \)). In a stepwise multiple regression model, ADMA level was the only independent determinant of flow-mediated dilatation (\( P=0.02 \)). In turn, race was the only independent determinant of ADMA levels (\( P=0.03 \)). Our findings indicate that circulating ADMA levels are significantly higher in healthy black African males than in white European males. This may contribute to the lower NO bioavailability and higher incidence of cardiovascular disease seen in black Africans. (Hypertension. 2007;49:873-877.)

Key Words: black African ■ white European ■ asymmetrical dimethylarginine ■ NO ■ endothelial function

Epidemiological studies have shown that American black Africans are at increased risk of cardiovascular disease and its complications compared with whites.1 This in part may be explained by an increased prevalence and severity in blacks of some risk factors for atherosclerosis. In particular, in black subjects, essential hypertension has a higher prevalence, earlier onset, and is associated with more severe end-organ damage, including left ventricular hypertrophy, renal failure, and stroke.1

However, conventional risk factors for cardiovascular disease do not account for all of this increased risk. A recent study demonstrated that black ethnicity is a strong and independent risk factor for the development of peripheral arterial disease, which was not explained by higher levels of diabetes, hypertension, or body mass index.2 The mechanisms underlying this ethnic predisposition to cardiovascular dysregulation remain unresolved.

A number of studies have demonstrated that black subjects have endothelial dysfunction,3–5 a key step in the initiation/progression of atherosclerotic vascular disease. A hallmark of endothelial cell dysfunction is a reduction in the bioavailability of the antiatherosclerotic signaling molecule NO.6 Longitudinal studies have demonstrated that a reduction in NO bioavailability is a predictor of accelerated atherosclerosis.7,8 NO is generated by a family of NO synthases from L-arginine in a reaction that requires oxygen, reduced nicotinamide-adenine dinucleotide phosphate, and essential cofactors, including tetrahydrobiopterin. NO bioavailability is principally determined by a reduction in its biosynthesis and/or inactivation by reactive oxygen species. Asymmetric dimethylargi-
nine (ADMA) has been shown to reduce NO bioavailability by competing with l-arginine as a substrate for endothelial NO synthase. In this study, we have assessed endothelial function and potential mechanisms leading to reduced NO bioavailability in young black African and white European males, who were rigorously matched for conventional risk factors for coronary artery disease.

**Methods**

**Subjects**

The study was carried out in the Cardiovascular Division at King’s College London School of Medicine. Fifty-eight healthy men were recruited to the study. Twenty-eight were white (European ancestry), and 30 were black (of African ancestry). Before the study, subjects were interviewed and screened for cardiac risk factors. Exclusion criteria were smoking, hypertension, hypercholesterolemia, diabetes mellitus, or cardiac disease. None of the subjects were on regular medication or had a recent acute illness. Each subject underwent detailed characterization including anthropomorphic measurements (weight [kilograms], height [meters], waist and hip circumference [centimeters], body mass index [kilograms per meter squared], and waist-to-hip ratio). Vascular response (endothelium-dependent function assessed by measuring percentage of flow-mediated dilation [FMD] and endothelium-independent function by percentage of vasodilator response to sublingual glyceryltrinitrate [GTN] in the brachial artery), full lipid profile (total cholesterol, triglyceride, low density lipoprotein cholesterol, and high density lipoprotein cholesterol), and levels of glucose, insulin, creatinine, ADMA, leptin, the inflammatory marker C-reactive protein (CRP), and a marker of systemic oxidative stress, isoprostane F2α, were assessed. None of the subjects were on regular medication or had a recent acute illness. Each subject underwent detailed characterization including anthropomorphic measurements (weight [kilograms], height [meters], waist and hip circumference [centimeters], body mass index [kilograms per meter squared], and waist-to-hip ratio). Vascular response (endothelium-dependent function assessed by measuring percentage of flow-mediated dilation [FMD] and endothelium-independent function by percentage of vasodilator response to sublingual glyceryltrinitrate [GTN] in the brachial artery), full lipid profile (total cholesterol, triglyceride, low density lipoprotein cholesterol, and high density lipoprotein cholesterol), and levels of glucose, insulin, creatinine, ADMA, leptin, the inflammatory marker C-reactive protein (CRP), and a marker of systemic oxidative stress, isoprostane F2α, were assessed.

**High-Resolution Ultrasonographic Studies of Brachial Artery**

Brachial artery vascular responses were measured using a 12.5-MHz linear array transducer (ATL), an HDI 5000-ultrasound machine, and a high-resolution ultrasonic vessel wall tracking system (HDI Laboratory, ATL). Scans were performed after an overnight fast in the morning and before vascular studies. The study had local ethics committee approval, and subjects gave written informed consent before recruitment.

**Laboratory Methods**

Fasting blood samples were drawn under standardized conditions stored at −80°C and analyzed in a blinded manner at the end of the study. Assays for lipid profile, glucose, insulin, and creatinine were performed in the hospital biochemistry department. ADMA was measured from serum samples by competitive ELISA (DLD Diagnostika GmbH) with a standard range from 0.1 to 5.0 μmol/L. The detection limit was 0.05 μmol/L. As described previously, intra-assay coefficients of variation were 7.5% at 0.81 μmol/L and 4.5% at 1.76 μmol/L, and interassay coefficients of variation ranged from 8.3% to 10.3%. The cross-reactivity of this immunoassay with arginine (<0.02%) and symmetric dimethylarginine (1.2%) is low. This method has been shown to have an excellent correlation with liquid-chromatography-mass spectroscopy and gas chromatography-mass spectroscopy (r = 0.984; P < 0.001). Leptin concentrations were measured using a quantitative sandwich ELISA technique (R&D Systems Europe). CRP was measured using high-sensitivity turbidimetric immunoassay (WAKO Chemicals) on the Cobas Mira Analyser (Roche Diagnostics). Total isoF2α levels in plasma were determined by immunoaffinity gas chromatography negative chemical ionization mass spectrometry as described previously. Creatinine clearance was estimated using the Cockcroft–Gault method. Estimated glomerular filtration rate (eGFR) was derived from creatinine levels as described by the Modification of Diet in Renal Disease Study and accounted for the age, sex, and racial origin of the subject.

**Results**

As outlined in Table 1 both ethnic groups were well matched for age, anthropomorphic measurements, cardiovascular risk factors and levels of glucose, insulin, and creatinine. Leptin, CRP, and isoF2α levels were also similar. ADMA levels were significantly higher (P = 0.03) in black Africans. Black Africans had lower creatinine clearance (P = 0.01) in comparison with white Europeans, but there was no difference in eGFR (P = 0.29).

**Factors Influencing Vascular Function**

Despite a similar cardiovascular risk profile, black African men had significantly lower FMD in comparison with white European men (FMD [% change]; black African: 5.2 ± 0.3, white European: 6.3 ± 0.4; P = 0.02). There was no difference in GTN-induced dilation between the 2 ethnic groups (GTN-induced dilation [% change]; black African: 19.7 ± 1.00, white European: 20.3 ± 0.1; P = 0.70).

Pearson correlation coefficients for the association between FMD and ADMA, leptin, CRP, isoF2α, creatinine clearance, eGFR, and baseline brachial artery diameter are summarized in Table 2. The only significant correlation was between FMD and ADMA levels (r = −0.31; P = 0.01; Figure). In a stepwise multivariate regression model (model 1),
ADMA level ($\beta = -0.31; P = 0.02$) was the only independent predictor of FMD (Table 3). None of the other variables entered into model 1 reached statistical significance (leptin: $\beta = -0.11, P = 0.39$; CRP: $\beta = -0.05, P = 0.69$; isoF2α: $\beta = -0.02, P = 0.86$; creatinine clearance: $\beta = 0.08, P = 0.54$; eGFR: $\beta = 0.19, P = 0.13$; baseline brachial artery diameter: $\beta = 0.09, P = 0.46$; and race: $\beta = -0.22, P = 0.10$).

### Discussion

The present study provides some novel insights into potential mechanisms underlying endothelium function in young black African males. Importantly, all of the subjects were closely matched for cardiovascular risk factors. Unlike previous studies, the present group of black African subjects was

### Factors Influencing ADMA Levels

Considering that ADMA was the only independent determinant of FMD, we examined factors influencing ADMA levels. Because a proportion of circulating ADMA is excreted by the kidneys, we examined the association between ADMA and creatinine clearance and eGFR. We found no association between these indirect measurements of renal function and ADMA. Furthermore, there was no correlation between CRP, isoF2α, and ADMA (Table 2). In a stepwise multivariate regression model (model 2), race ($\beta = 0.28; P = 0.03$) was the only independent predictor of ADMA levels (Table 3). There was no independent correlation between creatinine clearance ($\beta = 0.17; P = 0.20$), eGFR ($\beta = 0.15; P = 0.24$), and levels of CRP ($\beta = 0.14; P = 0.28$) and isoF2α ($\beta = 0.17; P = 0.18$) and ADMA in our cohort.

### Stepwise Multiple Regression Analysis to Assess Independent Determinants of Percentage of FMD (Model 1) and Plasma ADMA Levels (Model 2)

<table>
<thead>
<tr>
<th>Variable</th>
<th>% FMD $R$</th>
<th>$P$</th>
<th>ADMA Levels $R$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>-0.31</td>
<td>0.01</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.18</td>
<td>0.08</td>
<td>0.21</td>
<td>0.05</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.11</td>
<td>0.21</td>
<td>0.06</td>
<td>0.31</td>
</tr>
<tr>
<td>isoF2α</td>
<td>-0.08</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>0.06</td>
<td>0.33</td>
<td>0.19</td>
<td>0.10</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.13</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachial artery diameter</td>
<td>0.17</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only variables with a $P < 0.05$ were considered into the final fitted model. Model 1: dependent variable is percentage of FMD. Model 2: dependent variable is plasma ADMA levels.
young, normotensive, and, moreover, had similar body fat, blood pressure, insulin sensitivity, lipids, and inflammatory markers as white European subjects. Despite this, black Africans had blunted vasodilation in response to increased flow in the brachial artery.

We explored the potential influence of systemic inflammation (measured by CRP levels) and oxidative stress secondary to increased reactive oxygen species (assessed by measurement of isoF2α, a marker of systemic oxidative stress) on endothelial function. Our results were suggestive that, at this early stage of vascular dysfunction, there was no evidence of increased oxidative stress or systemic inflammation in black Africans. Similarly, leptin, a vasoactive peptide, also appeared to have no influence on vascular function. Furthermore, it is of relevance that we found no difference in smooth muscle responsiveness to GTN (endothelium-independent responses). To detect these subtle changes, vascular responsiveness to smaller incremental doses of GTN will need to be assessed.

ADMA, which is a competitive endogenous inhibitor of NO synthase, has emerged as a potentially important contributor to reduced NO bioavailability in a number of disorders characterized by cardiovascular disease, including aging, hypertension, diabetes, and hypercholesterolemia. Furthermore, 2 recent longitudinal studies, 1 of which included >1800 subjects, have demonstrated that ADMA is an independent predictor of future cardiovascular events. We have demonstrated that circulating ADMA levels are almost one third higher in healthy black African men compared with white European men. In a large study of patients with coronary artery disease, Schnabel et al demonstrated that the risk of cardiovascular events increased with increasing thirds of baseline ADMA. This observation is in support of a pathophysiologically relevant role for the different levels of ADMA seen in the present study. Also consistent with our data set, Houghton et al demonstrated that presence of the African race predicted an improvement in coronary endothelial function after supplementary L-arginine, which competes with ADMA. However, ADMA levels were not measured in this study.

Our results further demonstrated that, in healthy, young, normotensive black African males, conduit artery endothelial function is blunted. Endothelial function was independently associated with an increase in levels of ADMA. However, other potential determinants of endothelial function as assessed in our study (systemic inflammation, oxidative stress, leptin, creatinine clearance, eGFR, baseline brachial artery diameter, and race) appeared to have no independent influence on endothelial function. Our results highlight the importance of designing detailed future studies to examine the influence of acute and chronic L-arginine supplementation on levels of circulating ADMA and, hence, endothelial function in black Africans.

Considering that ADMA was the only independent determinant of endothelial function, we also examined factors influencing ADMA. Plasma ADMA levels were higher in black Africans in comparison with white Europeans. In multivariate analysis, race was the only independent determinant of plasma ADMA levels. Although ADMA is partially excreted by the kidneys, there was no independent association between indirect markers of renal function and plasma ADMA levels.

Measurement of ADMA
We used the recently introduced ELISA to measure ADMA rather than the more labor-intensive approaches using high-performance liquid chromatography. Although there has been some data suggesting that the ELISA may not correlate with high-performance liquid chromatography, there is a large body of data demonstrating a good correlation. Moreover, as discussed, 2 recent studies have demonstrated the prognostic use of ADMA measured using the ELISA in patients with coronary artery disease. Furthermore, our data in keeping with these studies demonstrated a significant correlation between ADMA measured using ELISA and FMD.

Study Limitations
NO bioavailability in the present study was assessed by measuring differences in the percentage of FMD in black African and white European men. Although there was a clear difference in FMD (a measurement of conduit artery endothelial function), we did not measure baseline flow. Baseline flow is principally dependent on microvascular function. Future studies exploring the mechanisms seen in the present report should also include assessment of microvascular function.

Circulating levels of ADMA have been shown to increase in renal failure. We, therefore, explored the relationship between creatinine clearance/eGFR and ADMA. Interestingly, we found no correlation between these indirect estimates of renal function and ADMA level in our cohort of healthy subjects. A potential explanation for this may be that, in subjects with normal renal function, clearance of plasma ADMA occurs in part by urinary excretion, with a substantial proportion of ADMA degraded by the intracellular enzyme dimethylarginine dimethylaminohydrolase after uptake from the circulation. However, creatinine clearance and eGFR are both indirect estimates of renal function. Assessing renal function using more sensitive/accurate techniques and its association with circulating ADMA levels would be worthwhile.

Perspectives
Young black African men, compared with white European men rigorously matched for cardiovascular risk factors, have significantly reduced NO bioavailability and, hence, greater endothelium-dependent brachial artery vascular dysfunction. This does not appear to be a result of increased oxidative stress, systemic inflammation, or conventional risk factors for atherosclerosis. Plasma ADMA, an endogenous competitive inhibitor of NO synthase, is significantly higher in black African than white European men and is an independent determinant of NO bioavailability. A number of studies have
shown that ADMA can be reduced by pharmacotherapy. For example, when glycemic control is improved by metformin, plasma ADMA levels are reduced in diabetic patients.25 In patients with insulin resistance, rosiglitazone improves insulin resistance and lowered plasma ADMA levels.26 Treatment of hypertensive patients with angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists reduces plasma ADMA levels.27,28 Therefore, ADMA may represent a novel target to improve NO bioavailability in African men at high risk of cardiovascular disease.

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Disclosures
None.

References
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