Defective Nitric Oxide Production and Functional Renal Reserve in Patients with Type 2 Diabetes Who Have Microalbuminuria of African and Asian Compared with White Origin

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Abstract. Diabetic nephropathy is a leading cause of end-stage renal failure. Its incidence is higher and is increasing in persons of Indo-Asian and African-Caribbean (African-Asian) compared with those of white origin. Nitric oxide deficiency is associated with progressive renal disease. It was hypothesized that differences in the capacity to increase glomerular filtration (functional renal reserve) would exist between these racial groups in relation to nitric oxide availability. Patients with type 2 diabetes of African-Asian (n = 9) and white (n = 9) origin with microalbuminuria were studied under euglycemic conditions. Glomerular filtration, renal plasma flow, and clearance of the stable metabolites of nitric oxide, nitrite, and nitrate were measured before and after a renal vasodilatory stimulus of a mixed amino acid intravenous infusion. There were no significant differences in age, duration of diabetes, and baseline glomerular filtration (57.1 [14.1] versus 55.8 [10.1] yr; P = 0.82, 14.5 [10.2] versus 9.1 [7.0] yr; P = 0.19 and 125.9 [30.9] versus 127.2 [44.6] ml/min per 1.73 m²; P = 0.94) between the African-Asian and white groups. Functional renal reserve, change in renal plasma flow, and percentage change in nitrate and nitrite clearance was significantly higher in the white compared with the African-Asian group (21.9 [45.7] versus −2.5 [28.2] ml/min per 1.73 m²; P = 0.043, 155.8 [205.9] versus −90.1 [146.0]; P = 0.03 ml/min per 1.73 m² and 26.7 [85.1] versus −44.7 [16.9] %; P = 0.013, respectively). The differences in functional reserve were not confounded after adjustment for diabetes duration (P = 0.034). The data suggest that these patients with type 2 diabetes of African and Asian origin lose functional renal reserve earlier in the evolution of nephropathy than whites. The differences appear to be due to defective nitric oxide production or bioavailability and might explain some of the propensity to develop end-stage renal disease.

Diabetic nephropathy is a leading cause of end-stage renal failure and premature death from cardiovascular disease, which is increasing worldwide (1,2). Susceptibility to and progression of this syndrome is not uniform. Nephropathy only develops in a subset of patients with diabetes; it clusters within families and has a striking predilection for certain racial groups (3,4). In the United Kingdom, patients of Indo-Asian and African-Caribbean origin share an increased susceptibility of developing end-stage renal failure that is four- to sixfold higher than that in whites (5). Furthermore, the variation in progression of renal disease among these racial groups appear to be independent of systemic BP control. It remains unknown whether these differences in renal disease susceptibility are linked to abnormalities of renal vasoregulation.

Nitric oxide (NO) is responsible for tonic vasodilatation, the regulation of systemic BP, and renal vascular resistance (6). A reduction in NO bioactivity is implicated in conditions associated with increased cardiovascular risk and renal failure. The capacity to increase glomerular filtration to renal vasodilatory stimuli is known as the functional renal reserve (FRR). This response is partly dependent on the action of NO and is preserved into old age but diminished by renal disease (7,8). Patients with diabetes and established clinical nephropathy display an absence of FRR (9). Impaired endothelial-dependent dilatation (10,11) and reduced vascular reserve (12) have been documented in established diabetes. Furthermore, hyperglycemia specifically and progressively impairs activation of the endothelial nitric oxide synthase pathway within the glomerulus (13). We considered that an abnormal renal hemodynamic phenotype would predate the decline in renal function in patients with a propensity to clinical diabetic nephropathy. Therefore, our aim was to determine whether racial differences exist...
in the renal vasodilatory response and its relationship to NO production.

Materials and Methods

Patients

Patient were recruited from the Whittington Hospital’s Diabetes Outpatient Clinics. Written informed consent was obtained from each patient, and the study was approved by the ethical committee of the Whittington Hospital Trust. Patients without a previous history of clinical proteinuria were referred to the “early nephropathy” clinic for further management of new-onset microalbuminuria. Microalbuminuria was diagnosed on the basis of having persistent albumin:creatinine (mg:mmol) ratios in sterile, early morning urine samples of ≥3 and confirmed by finding a urinary albumin excretion rate of between 30 and 300 mg/dL. A diagnosis of type 2 diabetes was based on an absence of ketosis or need for insulin within 1 yr of diagnosis. All patients had normal resting 12-lead electrocardiograms. Individuals with a history or clinical evidence of cardiovascular disease, malignancy, or other intercurrent illness were not included in this study. Smoking history was recorded.

Both parents of patients of black, African-Caribbean origin were native to either African or Caribbean countries (n = 5). Black, Indo-Asian patients and both of their parents originated from India, Pakistan, or Bangladesh (n = 4). For the purposes of this study, black, Indo-Asian, and African-Caribbean patients have been grouped together as “African-Asian.” Patients were confirmed as being white if both parents originated from either Western European or Mediterrane an countries (n = 9).

All vasoactive and antihypertensive medications were withdrawn for at least 5 wk before study. Ambulatory BP was measured over 24 h at 0.5 and 1.0 hourly intervals, respectively, during the wake and sleep hours (Spacelabs 90207, Spacelabs Medical, Washington, DC). Body surface area was calculated from weight in kg and height in m.

Retinopathy was assessed after dilated direct fundoscopy as either being present (background, preproliferative, proliferative, or evidence of laser therapy) or absent. Fasting serum total cholesterol, high-density lipoprotein cholesterol, and total triglyceride concentrations were determined by enzymatic methods. Serum creatinine was analyzed by a rate-reaction method. Glycosylated hemoglobin (HbA1c) and urinary albumin were measured by high-pressure liquid chromatography (HA 8121, Biomem, Berkshire, UK) and immunoturbidimetry, respectively.

Renal Hemodynamic Protocol

Patients were studied in the supine position after an overnight fast of 12 h and were not allowed to eat during the acute study. A 21-gauge venous cannula was inserted into an antecubital vein. A bolus dose of (35 mg/kg) inulin (Inutest, Pharma, Austria) and (8 mg/kg) paraminohippurate (MSD, Hoddesdon, UK) was given with constant infusions commenced at rates of 10 and 20 mg/min, respectively. A second cannula was inserted into the antecubital vein of the opposite arm for blood sampling. Plasma glucose was measured by the glucose oxidase method (Beckman Autoanalyser 2, Brea, CA) and maintained between 6 and 8 mmol/L throughout the study by use of a variable infusion of soluble insulin (Actrapid, Novo Nordisk, Basingstoke Hants, UK) and 5% dextrose. Hematocrit was assessed at baseline and at the end of the study. Baseline GFR and renal plasma flow (RPF) were determined from the mean of three 20-min clearances of inulin and paraminohippurate (PAH), respectively. After the baseline measurements, an amino acid mixture (Vamin, Pharmacia & Upjohn, Milton Keynes, UK) was infused (0.043 ml/kg per min) to stimulate renal blood flow. After an equilibration period of 60 min, the effect on RPF and GFR was determined from the mean of three clearances of inulin and PAH. Filtration fraction was calculated by dividing the GFR by RPF corrected for body surface area. Urine was collected by spontaneous voiding. To reduce the potential variability of clearance calculations by residual bladder urine, only studies that met the following criteria were evaluated: (1) the patient had no urinary symptoms suggestive of bladder dysfunction, (2) urine flow rates of ≥4 ml/min were maintained throughout the study, and (3) the intra-individual variation between the clearances was no more than 15% in each phase of the protocol.

Power Calculations and Statistical Analyses

In our preliminary studies, we found a mean increase in FRR of 20% in patients with diabetes with and without microalbuminuria. To have a 80% chance to detect a mean (SD) difference of 12% (3%) in FRR at the 5% (two-sided) level, a minimum of six patients would have to be studied in each racial group. Analyses were performed by use of SPSS 8.0 for Windows. Continuous variables were compared by use of parametric or nonparametric tests and associations tested with Spearman’s rank or Pearson’s test according to their distribution. Categorical variables were compared by use of χ² with continuity correction or Fisher’s exact test. For NOx clearance, racial group and duration in diabetes were used as covariates.

Results

The clinical and biochemical data of the patients studied in each group is shown in Table 1. The two groups were compa-
rable with respect to age and duration of diabetes. There were no differences in ambulatory BP and control of diabetes. The number of patients treated for diabetes with diet alone/oral hypoglycemic agents/insulin alone or insulin and oral hypoglycemic agents was similar in the African-Asian and white groups (2/1/6/0 versus 1/2/2/4; \(P = 0.47\), respectively). There were no current smokers. However, the number of ex-smokers was greater in the white (\(n = 3\)) compared with the African-Asian group (\(n = 1\); \(P = 0.57\)). Serum and urine concentrations of NOx were not statistically significantly different in the African-Asian and white groups (16.9 [6.8] versus 22.9 [11.1] \(\mu\)mol/L; \(P = 0.23\) and 273.6 [110.2] versus 509.8 [375.6] \(\mu\)mol/L; \(P = 0.29\), respectively). Renal function and renal hemodynamic parameters (GFR, RPF, and FF) were equivalent at baseline (Table 1).

Amino acid infusion significantly increased GFR and RPF from baseline in the whole group (126.6 [38.4] versus 136.9 [51.9] ml/min per 1.73 m\(^2\); \(P = 0.002\) and 548.1 [183.1] versus 581.0 [299.8] ml/min per 1.73 m\(^2\); \(P = 0.01\)). The magnitude of the change in GFR, RPF, and NOx clearance was significantly lower in the African-Asian compared with the white group (Figure 1), but plasma glucose was similar (6.9 [1.1] and 6.9 [0.6] mmol/L; \(P = 0.83\)) in both groups. Filtration fraction was significantly higher in the African-Asian group (Figure 2). The change in GFR (FRR) correlated positively with the change in NOx clearance (Figure 3). After adjustment for duration of diabetes, racial origin remained an independent determinant of NOx clearance (\(P = 0.034\)) and RPF (\(P = 0.045\)).

## Discussion

The increased predilection of patients with type 2 diabetes of Indo-Asian and African-Caribbean origin to develop nephropathy relative to white patients is unexplained. Hemodynamic factors that are considered central to the genesis of diabetic microangiopathy may play a role (16). We approached this question by investigating the effect of a mixed amino acid infusion on FRR, which has been shown to have a specific action on the renal vasculature. In the postabsorptive state, FRR can be completely abrogated by an intrarenal infusion of the nitric oxide synthase antagonist, NG-nomethyl L-arginine, which implicates its dependence on NO generation (17). Racial differences in the activity of the NO pathway have been previously described. Healthy individuals of African, compared with white, origin have reduced NO-mediated dilation of the forearm vasculature (18). However, the role, if any, and relevance of these hemodynamic factors to the racial differences in renal risk are unknown. Our data suggest that the alterations in the NO pathway may explain differences in diabetic renal disease phenotype.

Established diabetes is associated with endothelial dysfunction, which suggests depressed NO bioavailability. Some authors have found no change, whereas others have reported increases in NO production associated with hyperglycemia (19,20). However, depression in NO-dependent vasoregulation can be explained by its reduced bioavailability through inactivation. A metabolic consequence of persistent hyperglycemia is an increase in reactive oxygen species, which avidly inacti-

### Table 1. Demographic, clinical, biochemical, and baseline renal hemodynamic data of the African-Asian and white patients with type 2 diabetes who have microalbuminuria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>African-Asian</th>
<th>White</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of males (%)</td>
<td>9 (100)</td>
<td>8 (89)</td>
<td>0.87</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>57.1 (14.1)</td>
<td>55.8 (10.1)</td>
<td>0.82</td>
</tr>
<tr>
<td>Duration of diabetes mellitus (yr)</td>
<td>14.5 (10.2)</td>
<td>9.1 (7.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Retinopathy (%)</td>
<td>50</td>
<td>33</td>
<td>0.54</td>
</tr>
<tr>
<td>Number with positive smoking history (%)</td>
<td>1 (11)</td>
<td>3 (33)</td>
<td>0.56</td>
</tr>
<tr>
<td>Body surface area (m(^2))</td>
<td>1.75 (0.17)</td>
<td>1.80 (0.13)</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean systolic BP (mmHg)</td>
<td>144.0 (17.8)</td>
<td>137.6 (10.9)</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean diastolic BP (mmHg)</td>
<td>82.0 (6.5)</td>
<td>82.3 (8.8)</td>
<td>0.95</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 (1.5)</td>
<td>7.8 (1.3)</td>
<td>0.93</td>
</tr>
<tr>
<td>Fasting total cholesterol (mmol/L)</td>
<td>4.8 (1.1)</td>
<td>5.3 (1.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>Fasting high-density cholesterol (mmol/L)</td>
<td>1.2 (0.3)</td>
<td>1.3 (0.4)</td>
<td>0.65</td>
</tr>
<tr>
<td>Fasting total triglycerides (mmol/L)</td>
<td>1.5 (0.7)</td>
<td>1.9 (0.9)</td>
<td>0.41</td>
</tr>
<tr>
<td>Plasma creatinine ((\mu)mol/L)</td>
<td>103.5 (24.3)</td>
<td>92.0 (22.7)</td>
<td>0.29</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>6.6 (1.2)</td>
<td>7.2 (1.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>37.7 (2.6)</td>
<td>37.5 (4.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Albumin excretion rate (mg/d)(^a)</td>
<td>99.0 (56 to 161)</td>
<td>84.6 (50 to 240)</td>
<td>0.73</td>
</tr>
<tr>
<td>NOx clearance (ml/min per 1.73 m(^2))</td>
<td>122.9 (37.2)</td>
<td>100.3 (57.5)</td>
<td>0.40</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min per 1.73 m(^2))</td>
<td>125.9 (30.9)</td>
<td>127.2 (44.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min per 1.73 m(^2))</td>
<td>510.6 (135.8)</td>
<td>604.3 (246.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.24 (0.013)</td>
<td>0.21 (0.037)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\(^a\) Median (interquartile range).
vates NO (21,22). Furthermore, antioxidant therapy has been shown to improve endothelial-dependent function in patients with diabetes (23). It is of some note, therefore, that plasma lipid hydroperoxide, a marker of oxidative stress, is higher in patients with type 2 diabetes of African origin compared with white patients (24). Deficient NO production appears to be a common feature in renal insufficiency because of a variety of causes (25). Moreover, chronic inhibition of NO synthase is associated with increased progression of renal disease (26). An increase in the endogenous inhibitor of NO synthase, asymmetrical dimethylarginine occurs in renal failure (27). Furthermore, asymmetrical dimethylarginine levels are increased in patients prone to atherosclerosis with normal renal function (28). Therefore, several pathways may converge at the point of reduced NO bioactivity, which is associated with an increased risk of systemic and renal vascular disease.

A deficiency in NO might also contribute to pharmacologic differences in renal protection. The rate of decline in glomerular filtration is greater in patients of African descent compared with whites who have similar levels of systemic BP (29). Also, we have reported that the rate of increase in plasma creatinine is up to threefold higher in Indo-Asian compared with white subjects with type 2 diabetes who have equivalent BP and glycemic control (30). The angiotensin-converting enzyme class of antihypertensive drugs effectively retard the progression of diabetic nephropathy (31). Some, but not all, authors have shown this renal protective action to be independent of systemic BP control (32). Drugs from the angiotensin-converting enzyme class have been shown to improve FRR while concomitantly increasing the clearance of NOx (33). Moreover, recent experimental evidence has shown that increased production of NO as determined by the urinary excretion of NOx has a renoprotective action that is associated with in-

![Figure 1](image1)

**Figure 1.** Effect of mixed amino acid infusion on renal hemodynamic parameters and nitric oxide metabolite excretion in African-Asian (open bars) and white (stippled bars) patients with type 2 diabetes who have microalbuminuria corrected for body surface area of 1.73 m². (A) Change in GFR (ΔGFR) (*P = 0.043). (B) Change in renal plasma flow (ΔRPF) (*P = 0.03). (C) Percentage change in clearance of nitrite and nitrate (NOx) (*P = 0.013) (n = 8 in each group for this parameter).

![Figure 2](image2)

**Figure 2.** Effect of mixed amino acid infusion on filtration fraction (GFR/RPF) in African-Asian (open bars) and white (stippled bars) patients type 2 with diabetes who have microalbuminuria. *P = 0.02.

![Figure 3](image3)

**Figure 3.** Correlation between ΔGFR and clearance of NOx, corrected for body surface area of 1.73 m² after mixed amino acid infusion in patients with type 2 diabetes who have microalbuminuria (n = 16). *P < 0.001.
increased glomerular expression of endothelial nitric oxide synthase (34). Thus, depressed activity and/or a diminished capacity to elevate NO production in treated diabetic nephropathy might result in a relative acceleration of renal disease due to the failure to beneficially modulate intrarenal hemodynamics. In the present study, filtration fraction was decreased in white patients after amino acid infusion. This response suggests that filtration pressure rose as a result of an increase in afferent blood flow. The converse occurred in the African-Asian group.

In the present study, filtration fraction was decreased in white patients after amino acid infusion. This response suggests that filtration pressure rose as a result of an increase in afferent blood flow. The converse occurred in the African-Asian group. The association between FRF and NOx clearance suggests that defective NO-dependent afferent renal vasodilatation could contribute to these differences.

Our data are consistent with those of Stein et al. (18), which showed that a defect in the NO pathway accounts for abnormal vasoregulation in black compared with white patients, independently of disease status. However, neither study is able to confirm whether inherent factors are responsible for these differences. We limited the potential effect of dietary nitrate by studying patients after a 12-h fast, which has been shown not to affect endogenous NO metabolite levels (33). In these comparable patients, differences in NO production seem to underlie the renal hemodynamic abnormalities. This defect has effects on the renal vasculature that limits functional reserve and thereby could account for the acceleration of renal disease. The effect of long-term inhibition of NO production within the kidney is the development of glomerulosclerosis, in part because of the unmodulated action of angiotensin II (34–36). Of note, enhanced angiotensin II–dependent renal vasconstrictor function has been reported to occur in healthy, nondiabetic African Americans compared with whites (37). We have not demonstrated that NO bioaction is the basis for the differences, but this work provides support for the hypothesis that racial differences in renal vasoregulation in type 2 diabetes is related to depressed NO bioactivity that occurs in advance of renal failure.

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References


