Nitric Oxide Bioavailability and Its Potential Relevance to the Variation in Susceptibility to the Renal and Vascular Complications in Patients With Type 2 Diabetes

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OBJECTIVE — We compared the renal and systemic vascular (renovascular) response to a reduction of bioavailable nitric oxide (NO) in type 2 diabetic patients without nephropathy and of African and Caucasian heritage.

RESEARCH DESIGN AND METHODS — Under euglycemic conditions, renal blood flow was determined by a constant infusion of paraminohippurate and changes in blood pressure and renal vascular resistance estimated before and after an infusion of L-Ng-monomethyl-L-arginine.

RESULTS — In the African-heritage group, there was a significant fall in renal blood flow ($\Delta -46.0$ ml/min per 1.73 m^2; $P < 0.05$) and rise in systolic blood pressure ($\Delta 10.0$ mmHg [95% CI 2.3–17.9]; $P = 0.017$), which correlated with an increase in renal vascular resistance ($r^2 = 0.77$; $P = 0.004$).

CONCLUSIONS — The renal vasoconstrictive response associated with NO synthase inhibition in this study may be of relevance to the observed vulnerability to renal injury in patients of African heritage.

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The bioavailability of nitric oxide (NO) is central to the regulation of renovascular function and is reduced in established hypertension and diabetic nephropathy (1–3). Studies in rodents suggest that a deficiency of NO is an important susceptibility factor in the development of diabetes-related renal injury (4). It is unknown whether the differences in vulnerability to renal injury in diabetic patients of African heritage (5) versus Caucasians is related to NO bioactivity.

RESEARCH DESIGN AND METHODS

— We studied type 2 diabetic patients of African and Caucasian heritage. The patients in the African-heritage ($n = 9$) and Caucasian-heritage ($n = 11$) groups had similar distributions of sex, age, and duration of diabetes (male 75 vs. 70%, $P = 0.89$; mean ± SD age 53.3 ± 7.2 vs. 55.2 ± 4.6 years, $P = 0.50$; and duration 10.3 ± 10.7 vs. 6.8 ± 6.4 years, $P = 0.37$, respectively). Systolic blood pressure and diastolic blood pressure were 124.4 vs. 122.1 mmHg ($P = 0.75$) and 77.0 vs. 76.1 mmHg ($P = 0.81$), respectively. The patients were naïve to antihypertensive therapy, and equal numbers in each group received metformin ($n = 6$) and insulin ($n = 2$).

A1C and urinary albumin were measured by high-pressure liquid chromatography (HA 8-121; Biomen, Berkshire, U.K.) and immunoturbidimetry, respectively. Serum creatinine was analyzed by a rate-reaction method. Estimated creatinine clearance was calculated from the Cockcroft-Gault formula. Microalbuminuria was excluded on the basis of three consecutive albumin-to-creatinine ratios <3 mg/mmol in sterile, early-morning urine samples and a urinary albumin excretion rate <30 mg/day.

Renal plasma flow (RPF) was measured by the constant infusion method (6,7). A bolus dose of 8 mg/kg paraminohippurate (Merck, Sharp & Dohme, Hoddesdon, U.K.) was given with a 20 mg/min infusion. After a 90-min equilibration period, the concentration of the infusate was multiplied by the infusion flow rate and divided by the mean of duplicate plasma samples at this and subsequent time points. Plasma paraminohippurate was assayed after deproteinizing the samples with 6% trichloroacetic acid for 10 min at 70°C and sequentially adding sodium nitrite, ammonium sulfamate, and N-1-naphthylethylenediamine using a Cobas Mira (Roche, Lewes, U.K.).

After initial equilibration, an amino acid mixture (Vamin; Pharamcia & Upjohn, Milton Keynes, U.K.) was infused ($0.043 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). RPF was assessed 80 min later, and then L-NMMA (Clinalfa, Laufelfingen, Switzerland) was begun at the nonpressor dose of 20 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Both infusions were continued for a further 20 min, after which a final RPF measurement was made.

During the studies, blood pressure was monitored automatically (Dinamap; Critikon, Basingstoke, U.K.), and whole blood was sampled from a venflon in a hand vein to measure glucose by the oxidase method (One Touch; Lifescan, High Wycombe, U.K.) every 10 min. Mean arterial pressure (MAP) was calculated as the diastolic blood pressure plus one-third of the pulse pressure. Renal blood flow (RBF) was calculated by dividing the

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RPF by 1 hematocrit and renal vascular resistance (RVR) by dividing MAP by RBF. The study was approved by the ethics committee of the Whittington Hospital National Health Service Trust.

Statistical analysis

Analyses between or within the groups were performed using SPSS for Windows (version 10; SPSS, Chicago, IL). Continuous variables were compared with parametric or nonparametric tests and associations tested with Spearman's rank correlation test or Pearson's X^2 test according to their distribution. Categorical variables were compared using a χ^2 test with continuity correction or Fisher's exact test. Clearance and RPF measurements were corrected for a body surface area of 1.73 m^2. Data are expressed as means ± SD unless otherwise stated.

RESULTS — Comparative baseline measurements of RPF and systolic and diastolic blood pressures were similar between the African-heritage and Caucasian-heritage groups (RPF 533.7 ± 174.7 vs. 565.3 ± 260.8 ml/min per 1.73 m^2, $P = 0.78$; systolic 124.9 ± 23.7 vs. 121.6 ± 12.3 mmHg, $P = 0.29$; and diastolic 77.1 ± 9.5 vs. 76.3 ± 5.7 mmHg, $P = 0.81$, respectively). There were no differences in creatinine clearance or median urinary albumin excretion rate (93.7 ± 19.9 vs. 98.9 ± 19.5 ml/min per 1.73 m^2, $P = 0.57$, and 12.6 [4.1–25.0] vs. 14.0 [interquartile range 8.5–24.1] mg/day, $P = 0.79$). Averaged blood glucose was similar (6.7 ± 0.9 vs. 7.4 ± 0.9 mmol/l; $P = 0.14$). A1C was lower in the African-heritage than in the Caucasian-heritage group (6.8 ± 0.69 vs. 8.0 ± 0.94%; $P = 0.005$).

The L-NMMA infusion was associated with significant changes in systolic blood pressure in the African-heritage group (Fig. 1). Relative to the baseline and post–amino acid measurements, there was a mean rise of 10.0 mmHg (95% CI 2.3–17.9; $P = 0.017$) and 7.3 mmHg (1.0–13.7; $P = 0.03$), respectively, in the African-heritage group and 4.3 mmHg (−1.8 to 10.4; $P = 0.23$) and 2.4 mmHg (−3.5 to 8.3; $P = 0.38$) in the Caucasian-heritage group. Final blood pressure was higher in the African-heritage group (137.5 ± 9.0 vs. 123.4 ± 14.2 mmHg; $P < 0.05$) and was associated with a fall in RBF (Δ−46.0 ml/min per 1.73 m^2; $P < 0.05$) and a rise in RVR (from 0.12 ± 0.06 to 0.14 ± 0.04 mmHg ml/min per 1.73 m^2; $P = 0.036$). The changes in RVR correlated with MAP ($r^2 = 0.77$; $P = 0.004$). Renal hemodynamic measures were unchanged in the Caucasian-heritage group.

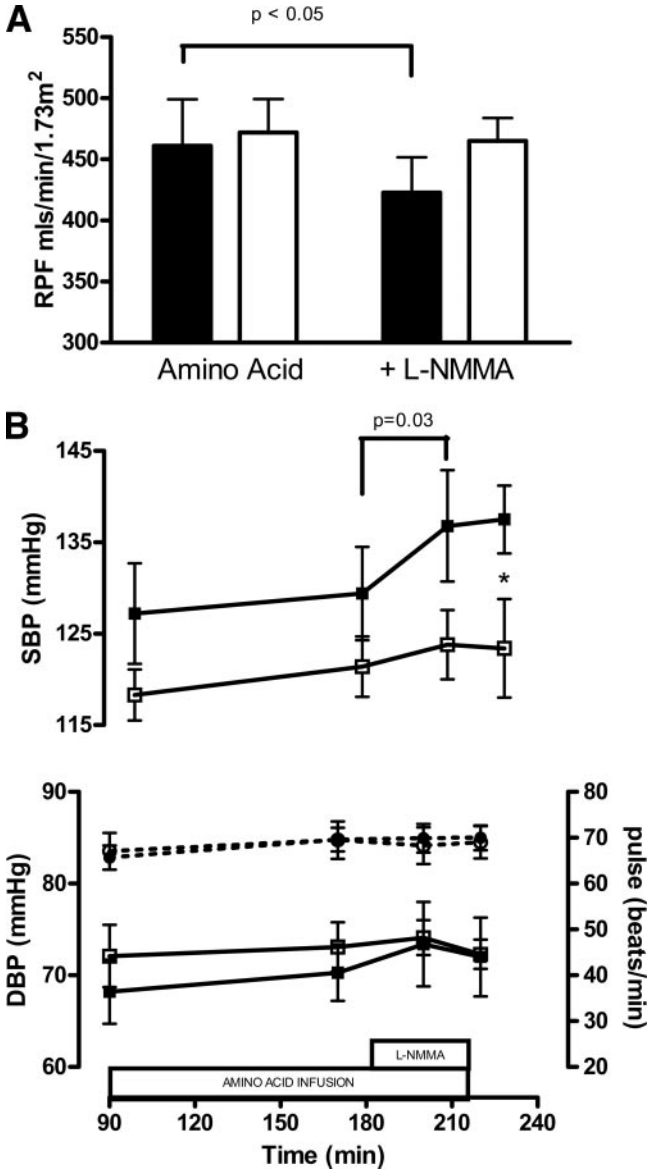

Figure 1—*Data are means ± SD.* A: *RPF measured at end of infusion with amino acid and after co-infusion of amino acid with L-NMMA (+L-NMMA) in patients with type 2 diabetes, which fell significantly in the African-heritage group compared with the Caucasian-heritage group. B: Profile of systolic (SBP) and diastolic (DBP) blood pressure and pulse rate (dashed line) in patients with type 2 diabetes of African and Caucasian heritage during phases of the hemodynamic studies. In the African-heritage group, SBP rose significantly in response to L-NMMA and was higher at the end of study than that for the Caucasian group.* ■, *African-heritage group;* □, *Caucasian-heritage group;* ●, *pulse of African-heritage group;* ○, *pulse of Caucasian-heritage group.* $P < 0.05$ *after L-NMMA infusion.*

CONCLUSIONS — In this study, patients without hypertension or renal disease of African heritage had an increased sensitivity to the renal vasoconstrictive effect of NO synthase (NOS) inhibition. These data suggest that a reduction in NO bioavailability may adversely affect autoregulatory processes that could potentially increase vulnerability to renal damage (8).

We used the amino acid infusion to optimize renal blood flow and suppress tubuloglomerular feedback as a contributor to vasoconstriction. The myogenic component of the autoregulatory response is attenuated by NO (9). Therefore, the reduction in renal blood flow that we observed was probably due to an effect of NOS inhibition on the renovascular smooth muscle.

Early in the course of diabetes, NO production is necessary to forestall a rise in blood pressure. Hypertension is associated with the generation of NO-quenching free radicals and is a prerequisite for the development of renal disease (10–12). Furthermore, the renal expression of NOS in patients with diabetes is related to the degree of vasculopathy (13). It could therefore be considered that upregulation of NO production in patients of African heritage is related to a mechanism that opposes an enhanced vasoconstrictor tendency. Although consistent with experimental studies, these outcomes require caution before being generalized. Confirmatory studies in patients with and without diabetes with greater power and the evaluation of the role of vasoconstrictive cytokines, angiotensin II, or endothelin-1 as potential contributors to this hemodynamic response are now required.

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No potential conflicts of interest relevant to this article were reported.

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