Poor Glycemic Control Is Related to Increased Nitric Oxide Activity Within the Renal Circulation of Patients With Type 2 Diabetes

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OBJECTIVE—Experimental studies have shown that glucose releases endothelial nitric oxide (NO) and that NO contributes to renal hyperperfusion in models of diabetes. To examine whether this translates into the human condition, we studied the relationship between glycemic control and renal NO activity in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 113 patients with type 2 diabetes and a wide range of HbA_{1c} concentrations were included. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined by constant infusion input clearance. Functional NO activity in the renal circulation was determined as change of RPF to infusion of the NO synthase (NOS) inhibitor N(G)-monomethyl-L-arginine (L-NMMA) (4.25 mg/kg). As additional markers, we measured urinary excretion of NO (UNOx) and L-arginine–to–asymmetrical dimethylarginine (ADMA) ratio in plasma.

RESULTS—Subjects within the highest tertile of HbA_{1c} concentration had increased RPF (low, medium, and high tertiles 576 ± 17 vs. 585 ± 22 vs. 627 ± 33 mL/min/m^2, $P = 0.05$ by one-way ANOVA), while GFR was similar across tertiles. The response of RPF to NOS blockade was augmented in subjects with higher HbA_{1c} levels (−55 ± 7 vs. −64 ± 8 vs. −86 ± 8 mL/min, $P = 0.04$ by one-way ANOVA). Further, L-arginine–to–ADMA ratio and UNOx were increased in subjects with higher HbA_{1c} levels.

CONCLUSIONS—In line with experimental evidence, we could demonstrate in humans that poor glycemic control is related to higher NO activity and hyperperfusion of the kidney. The renal NO system may thus be a novel therapeutic target for improving renal hemodynamics in patients with diabetes.

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The incidence of end-stage renal disease owing to diabetic nephropathy is increasing in developed countries (1). In order to reduce the burden of end-stage diabetic kidney disease, targeting glomerular hyperfiltration and hyperperfusion, early hemodynamic abnormalities that have been linked with greater risk of developing albuminuria and loss of renal function over time (2,3), may be an attractive therapeutic option.

Others and we have shown that nitric oxide (NO) is an important regulator of renal hemodynamics in humans (4–6). Experimental studies have demonstrated that increased production of NO in the kidney contributes to the renal hemodynamic alterations in models of type 1 and type 2 diabetes (7–12). As a pathogenetic factor, hyperglycemia has been shown to stimulate acute release of NO from cultured endothelial cells (13,14), including endothelial cells derived from the glomerulum (15).

In human subjects with diabetes, data on the role of NO for renal hemodynamics are very limited. A few studies are available that have assessed NO production with a biochemical approach. Hiragushi et al. showed that in subjects with type 2 diabetes, urinary NO (UNOx) excretion rates were higher in those with increased glomerular filtration rate (GFR) versus those with normal GFR (16). Additional studies suggested that it is the hyperglycemia that drives increased NO production associated with glomerular hyperfiltration (17,18).

Using a much more direct way of assessing the functional contribution of NO to renal hemodynamics, Cherney et al. (19) studied the renal response to pharmacological NO synthase (NOS) inhibition in subjects with type 1 diabetes without complications. NOS inhibition led to a significantly greater decline of GFR and renal plasma flow (RPF) in hyperfiltering versus the normofiltering subjects with type 1 diabetes.

The role of NO in renal hemodynamics of subjects with type 2 diabetes, a more heterogenous group of subjects with regard to concomitant diseases and vascular risk factors, and the influence of glycemic control have not been studied. To this end, we examined renal hemodynamic responses to pharmacological NOS inhibition across a wide range of HbA_{1c} levels in a large cohort of subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patient selection

Patients who were treated in our outpatient clinic for type 2 diabetes or participated in our training program for patients with type 2 diabetes were asked to take part in the current study when they fulfilled the following inclusion criteria: age between 30 and 75 years and office blood pressure (BP) <180/110 mmHg. Exclusion criteria were impaired renal function defined by a serum creatinine >1.3 mg/dL in men and >1.2 mg/dL in women; overt albuminuria >300 mg/day; any other severe renal, hepatic, or cardiovascular disease; current

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antihypertensive medication or lipid-lowering therapy; insulin therapy; current use of oral contraceptives or estrogen replacement therapy; and active smoking. BP during screening was recorded as the average of three measurements after 5 min of rest. All patients gave their written informed consent prior to study inclusion. Patients who were treated with an oral hypoglycemic agent were asked to withhold the morning dose on the day of the clearance study. A sample size of 35 patients in each group was required to exclude a difference in the response of RPF to N(G)-monomethyl-L-arginine (L-NMMA) of 20 mL/min/m^2 at an SD of 25 by one-way ANOVA and at a power of 85% and a *P* value of < 0.05. The Clinical Investigations Ethics Committee of the University of Erlangen-Nürnberg approved the study protocol.

Infusion protocol

Systemic hemodynamic parameters (i.e., BP and heart rate) were monitored with an oscillometric device (Dinamap 1846 SX; Criticon, Norderstedt, Germany). Renal hemodynamic parameters were determined by the constant infusion input clearance technique with inulin and sodium *p*-aminohippurate (Clinalfa, Basel, Switzerland) for GFR and RPF, respectively, as previously described (4,5,20).

Briefly, after bolus infusion of inulin and sodium *p*-aminohippurate over 15 min and a subsequent constant infusion over 105 min, a steady state between input and renal excretion of the tracer substances is reached (4,5,20). Then, L-NMMA is administered as a bolus infusion (3 mg/kg i.v. over 5 min) followed by constant infusion (1.25 mg/kg i.v. over 25 min) to determine the functional activity of NO in the renal circulation (4,5,20). As a safety measure, L-arginine (L-arginine hydrochloride 6%; University Hospital Pharmacy, Erlangen, Germany) is then administered at a dose of 100 mg/kg i.v. over 30 min to reverse NOS inhibition by excess substrate availability (data not shown). Blood samples to determine inulin and *p*-aminohippurate concentrations were drawn at 0, 120, and 150 min. During the last 5 min of each infusion step, BP was monitored every minute, and the mean of these measurements is given. Filtration fraction (FF) was calculated as GFR/RPF. All renal hemodynamic parameters were standardized to body surface area.

Laboratory measurements

Laboratory tests were performed at study inclusion to test for inclusion and exclusion criteria. Blood glucose concentration was measured in serum by use of the hexokinase reaction. Measurements of *p*-aminohippurate and inulin were performed after completion of the study from blood samples centrifuged immediately at 4°C and stored at −21°C. *p*-aminohippurate was measured by the method of Smith et al. (21); inulin was determined indirectly with an enzymatic method after conversion to fructose. Serum L-arginine and asymmetrical dimethylarginine (ADMA) concentrations were obtained using high-performance liquid chromatography measurements. The L-arginine–to–ADMA ratio was then calculated as an index of NOS function (22). Urinary NO measurements were performed with the Griess reaction (Nitric Oxide Metabolite Detection Kit; Cayman Europe, Tallinn, Estonia). Each blood sample was measured in duplicate with a coefficient of variation of <5%.

Statistics

Analyses were performed using SPSS Software (PASW statistics 20.0; IBM, Ehningen, Germany). After confirmation of normal distribution by Kolmogorow-Smirnov tests, one-way ANOVA was used to compare parametric clinical parameters, whereas Kruskall-Wallis tests were used to compare nonparametric data. Categorical data were compared with the χ^2 test. Data are given as mean ± SEM, and a *P* value <0.05 (two-sided) was considered statistically significant.

RESULTS

Clinical parameters

A total of 113 subjects with type 2 diabetes were enrolled in the study. HbA_{1c} values ranged from a minimum of 5.2% (33 mmol/mol) to a maximum of 9.7% (83 mmol/mol). Subjects were classified

Table 1—*Clinical characteristics according to HbA_{1c} tertiles*

Parameter	Low HbA_{1c}: 5.2–6.4% (33–46 mmol/mol)	Medium HbA_{1c}: 6.4–7.3% (46–56 mmol/mol)	High HbA_{1c}: 7.3–9.7% (56–83 mmol/mol)	*P*
n	38	38	37	
HbA_{1c}				
%	6.0 ± 0.1	6.8 ± 0.1	8.1 ± 0.1	<0.001
mmol/mol	42 ± 0.3	51 ± 0.3	65 ± 0.3	<0.001
Serum glucose (mg/dL)	137 ± 7	168 ± 7	210 ± 10	<0.001
Age (years)	58 ± 1	60 ± 1	61 ± 1	0.246
Sex (male/female)	25/13	26/12	24/13	0.914
BMI (kg/m^2)	30 ± 1	30 ± 1	30 ± 1	0.812
SBP (mmHg)	148 ± 3	150 ± 2	152 ± 3	0.474
DBP (mmHg)	88 ± 2	88 ± 1	84 ± 2	0.295
HDL cholesterol (mg/dL)	51 ± 2	45 ± 2	45 ± 2	0.069
LDL cholesterol (mg/dL)	134 ± 6	138 ± 6	136 ± 6	0.901
Triglycerides (mg/dL)	192 ± 17	218 ± 25	220 ± 23	0.583
Serum creatinine (mg/dL)	0.8 ± 0.03	0.8 ± 0.03	0.8 ± 0.04	0.375
Serum urea (mg/dL)	35 ± 1	32 ± 1	37 ± 3	0.170
Urinary sodium (mmol/24 h)	117 ± 54	117 ± 49	139 ± 77	0.244
UACR 24 h (mg/g creatinine)	9 ± 3	10 ± 3	39 ± 18	0.063
UACR spot (mg/g creatinine)	21 ± 7	28 ± 11	63 ± 20	0.069

UACR 24 h, urinary albumin-to-creatinine ratio from 24-h urine collection; UACR spot, urinary albumin-to-creatinine ratio from spot urine collection.

into tertiles according to their HbA_{1c} concentration (Table 1). The HbA_{1c} concentration cutoff value between the low and the medium HbA_{1c} groups was 6.4% (46 mmol/mol). The cutoff value between the medium and the high HbA_{1c} groups was 7.3% (56 mmol/mol).

The greater the HbA_{1c} concentration, the greater the fasting blood glucose level. Clinical parameters such as age, BMI, sex distribution, and BP were similar between groups. There was a trend toward greater urinary albumin excretion rates (but still within the microalbuminuric range) in groups with higher HbA_{1c} levels.

Indirect markers of renal NO production

UNOx was greatest in those within the highest tertile of HbA_{1c} concentration (Fig. 1). Bonferroni corrections revealed significant differences in UNOx between the low versus the high HbA_{1c} groups ($P < 0.05$) and the medium versus high HbA_{1c} groups ($P < 0.05$).

Plasma L-arginine concentrations (32 ± 8 vs. 52 ± 11 vs. 56 ± 15 μmol/L, $P = 0.14$) and ADMA levels (0.70 ± 0.3 vs. 0.67 ± 0.03 vs. 0.65 ± 0.03 μmol/L, $P = 0.21$) were similar across tertiles. However, the ratio of plasma L-arginine to ADMA was significantly increased in those within the highest HbA_{1c} tertile (Fig. 2). Bonferroni corrections revealed significant differences in the L-arginine–to–ADMA ratio between the low versus the high HbA_{1c} groups ($P < 0.05$).

Direct assessment of functional NO activity in the renal circulation

Baseline systolic BP (SBP), diastolic BP (DBP), and HR were similar across the three HbA_{1c} tertiles (Table 2). Infusion of L-NMMA at a dose of 4.25 mg/kg body wt increased SBP and DBP, while HR decreased in all three groups. There was no difference in the magnitude of the changes in SBP, DBP, and HR across the three groups (all n.s.).

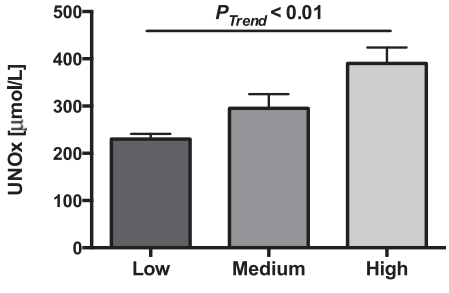

Figure 1—*UNOx across HbA_{1c} tertiles.*

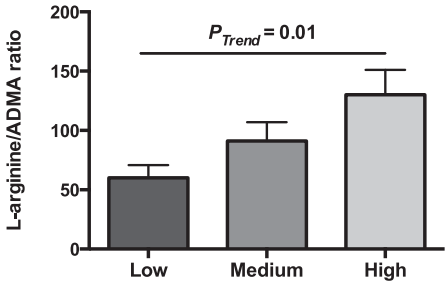

Figure 2—*L-Arginine–to–ADMA ratio across HbA_{1c} tertiles.*

Baseline RPF was greatest in the subjects within the highest tertile of HbA_{1c}. GFR was not different across HbA_{1c} tertiles, but there was a trend toward slightly lower FF in those with higher HbA_{1c} concentrations ($P = 0.08$). We subsequently assessed renal hemodynamic response to NOS inhibition, reflecting functional NO activity within the renal circulation. RPF decreased significantly in response to the infusion of L-NMMA, while GFR and FF increased in all three tertiles. However, the reduction of RPF with L-NMMA infusion was greatest in subjects within the highest HbA_{1c} tertile, indicating greater functional NO activity in the renal circulation (Fig. 3). This difference across HbA_{1c} tertiles persisted when baseline RPF values were adjusted for ($P = 0.045$). Further, there was a significant correlation between HbA_{1c} levels and response to L-NMMA ($r = -0.264$, $P = 0.009$). Bonferroni corrections revealed significant differences in the response of RPF to L-NMMA between the low versus the high HbA_{1c} tertile ($P < 0.05$). After L-NMMA infusion, RPF was similar across HbA_{1c} tertiles.

CONCLUSIONS—In this study, we found that poor glycemic control is related to higher NO activity and hyperperfusion of the kidney in patients with type 2 diabetes. The renal NO system may thus be a novel therapeutic target for improving renal hemodynamics.

Experimental studies have shown that NO is a potent direct vasodilator of afferent and efferent arterioles (23). Further, NO inhibits tubuloglomerular feedback–mediated vasoconstriction of afferent arterioles (24). Clearance studies have confirmed that NO is also an important mediator of renal hemodynamics in humans (4,5). Therefore, we hypothesized that increased NO production could underlie the hyperperfusion state in human subjects with diabetes, as suggested by a number of animal studies (7–12).

In a first study addressing this issue in humans, Cherney et al. (19) analyzed the response of renal hemodynamics to pharmacological NOS inhibition in 37 subjects with type 1 diabetes and in 21 healthy control subjects. For the analysis of the renal response to NOS inhibition, the 37 diabetic subjects were divided into those with glomerular hyperfiltration (GFR >135 mL/min) and those with normal GFR. L-NMMA led to a decline of GFR in the hyperfiltering diabetic subjects but neither in the diabetic subjects with normal GFR nor in healthy control subjects. Furthermore, the decline of RPF was exaggerated in the hyperfiltering group versus the other two groups. However, studies in patients with type 2 diabetes, a more heterogeneous group of patients owing to older age and greater number of concomitant cardiovascular risk factors, have been lacking to date.

In the current study, subjects with type 2 diabetes and increased HbA_{1c} concentrations, reflecting poorer glycemic control, were characterized by higher baseline RPF. There was a trend toward lower FF in those with higher HbA_{1c} levels ($P = 0.08$). This would be in keeping with experimental data of vasodilation of both afferent and efferent arterioles and would explain why glomerular filtration pressure and thus GFR remained unchanged with higher HbA_{1c} values (25). This also fits with experimental evidence that NO is a vasodilator of both afferent and efferent arterioles (23). Indeed, as the main result of our study, the reduction of RPF and thus the contribution of NO to renal perfusion was greatest in subjects within the highest tertile of HbA_{1c} levels. To confirm the increased functional contribution of NO to renal perfusion as demonstrated by the renal clearance technique, we performed two additional assays of NO production. Increased HbA_{1c} was associated with an increase in the ratio of L-arginine to ADMA, which has been suggested as a marker of endothelial function and NO production (22). In addition to increased L-arginine/ADMA levels, increased NO activity in subjects with higher HbA_{1c} levels was also supported by the finding of increased UNOx, which at least in part depends on renal production of NO (26).

Our results of an increased NO production differ from the result of a reduction

Table 2—*Systemic and renal hemodynamics before and after infusion of L-NMMA according to HbA_{1c} tertiles*

	Low HbA_{1c}: 5.2–6.4% (33–46 mmol/mol), *n* = 38		Medium HbA_{1c}: 6.4–7.3% (46–56 mmol/mol), *n* = 38		High HbA_{1c}: 7.3–9.7% (56–83 mmol/mol), *n* = 37	
	Baseline	L-NMMA	Baseline	L-NMMA	Baseline	L-NMMA
BP						
SBP (mmHg)	141 ± 2	151 ± 2*	142 ± 2	152 ± 3*	145 ± 2	159 ± 3*
DBP (mmHg)	80 ± 2	87 ± 2*	79 ± 1	85 ± 2*	79 ± 2	85 ± 2*
HR (bpm)	63 ± 2	59 ± 2*	66 ± 2	62 ± 2*	65 ± 2	60 ± 2*
Renal hemodynamics						
RPF (mL/min/m^2)	576 ± 17	520 ± 17*	585 ± 22	530 ± 24*	627 ± 33#	554 ± 32*§
GFR (mL/min/m^2)	133 ± 3	138 ± 4*	133 ± 4	138 ± 4*	132 ± 4	135 ± 4*
FF (%)	23 ± 1	27 ± 1*	23 ± 1	27 ± 1*	21 ± 1	25 ± 1*

Systemic and renal hemodynamics before and after infusion of L-NMMA according to HbA_{1c} tertiles. *$P < 0.05$ for the comparison of L-NMMA vs. baseline by paired *t* test within HbA_{1c} tertiles. #$P < 0.05$ for the comparison of baseline RPF across HbA_{1c} tertiles by one-way ANOVA. §$P = 0.04$ for the comparison of the change between L-NMMA and baseline across HbA_{1c} tertiles by one-way ANOVA.

in cyclic guanosine monophosphate levels in female subjects with type 1 diabetes upon acute exposure to hyperglycemia during glucose infusion (27). However, the physiological effects of acutely induced hyperglycemia versus those of chronic hyperglycemia are difficult to compare (e.g., due to differences in acute shear stress). Furthermore, cyclic guanosine monophosphate is stimulated not only by NO but also by other hormones such as the natriuretic peptides. In keeping with our results, and using a similar methodology, the already mentioned study by Cherney et al. (19) also found increased, rather than decreased, NO production associated with increased renal perfusion.

Of note, we have shown that increased HbA_{1c} is related to renal hyperperfusion and increased NO production but not with renal hyperfiltration. "Hyperfilterers" may in fact be a distinct subgroup of subjects, characterized by increased cyclooxygenase contribution to afferent arteriolar tone (28,29). Thus, the factors that determine renal hyperperfusion appear to differ from those that determine renal hyperfiltration (e.g., NO vs. prostaglandins).

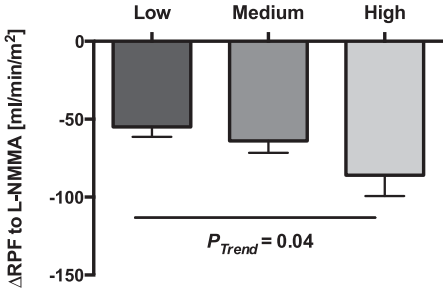

Figure 3—*Change of RPF in response to L-NMMA infusion across HbA_{1c} tertiles.*

Our study has several limitations. Since L-NMMA is an unselective NOS inhibitor, the precise isoform of NOS responsible for increased NO production in subjects with high HbA_{1c} values remains to be identified. Some cell culture and some animal studies suggest that NOS2 (inducible NOS) is upregulated (8,11,12,30), while others suggest that NOS3 (endothelial NOS) is the responsible isoform (10). Another limitation is that glycemia in the majority of our patients was rather well controlled, and perhaps even greater differences between groups would have been observed with a larger number of less well-controlled patients included. Further, to avoid confounding effects on renal endothelial function and hemodynamics, we excluded subjects with more severe hypertension (≥180/110 mmHg), those on antihypertensive or lipid-lowering therapy, and those on insulin treatment. This may have limited generalizability to a larger number of subjects with type 2 diabetes.

A number of experimental and clinical studies have shown that improved glycemic control ameliorates alterations of renal hemodynamics (31). Whether this is related to normalization of renal NO production remains to be investigated in future. Furthermore, additional therapies that target abnormal renal hemodynamics in subjects with type 1 and type 2 diabetes would be welcome in the clinical setting, as strict glycemic control is not always a realistic treatment target in every patient. We have previously shown that antioxidant treatment with folic acid is able to normalize increased NOS dependence of renal vascular tone in subjects with the metabolic syndrome (20). As an additional treatment option, future studies need to address whether antioxidant treatment can improve renal hemodynamics by normalizing renal NO activity in subjects with diabetes mellitus.

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

M.P.S. designed the study, researched data, and wrote the manuscript. C.O. researched data and contributed to the discussion. S.S., I.K., and S.F. researched data. R.E.S. designed the study and wrote and finally approved the manuscript. R.E.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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