Gender is related to alterations of renal endothelial function in type 2 diabetes

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Abstract

Background. Gender has been shown to affect endothelial function of the forearm circulation in patients with type 2 diabetes, but data on the renal circulation are lacking. We hypothesized that renal vascular nitric oxide (NO) availability is higher, and oxidative stress lower, in female compared to male patients with type 2 diabetes.

Methods. In 41 male and 39 female patients with type 2 diabetes, renal plasma flow (RPF) was determined by constant infusion input clearance at baseline and following infusion of the NO synthase inhibitor N(G)-monomethyl-L-arginine (L-NMMA, 4.25 mg/kg) to assess basal renal vascular NO availability. After a subsequent infusion of L-arginine (100 mg/kg) to restore baseline conditions, vitamin C (45 mg/kg) was co-infused to determine levels of oxidative stress in the renal circulation.

Results. Baseline renal haemodynamics were similar between genders. L-NMMA-induced renal vasoconstriction was more pronounced in females compared to males (−89 ± 69 versus −60 ± 52 ml/min/1.73 m², P = 0.03). After administration of L-arginine to restore baseline perfusion, the co-infusion of vitamin C led to a lesser increase of RPF in females than in males (+37 ± 86 versus +60 ± 52 ml/min/1.73 m², P = 0.05).

Conclusions. Our data demonstrate that NO availability in the renal circulation is greater in female than in male patients with type 2 diabetes that is associated with reduced levels of oxidative stress in females. The role of this gender-related difference in renal endothelial function for the initiation and progression of diabetic nephropathy should be addressed in future studies.

Keywords: endothelium; gender; kidney; nitric oxide; oxidative stress
Introduction

Over the last decades, the pivotal role of the endothelium in preserving vascular integrity has attracted increasing attention. Studies in humans have shown that an impairment of endothelium-mediated, nitric oxide (NO)-dependent vasodilation in the forearm and in the coronary circulation predicts cardiovascular events [1,2]. Similarly, there is convincing experimental evidence that an impairment of renal endothelial function can predict the progression of renal disease [3]. In humans, the direct assessment of renal endothelial function by intra-arterial administration of drugs into the kidney is not feasible, and thus, a clearance method has been established to assess the contribution of NO and oxidative stress to renal haemodynamics [4–6].

Using this approach, it has been shown that oxidative stress is increased in the renal circulation of patients with type 2 diabetes [5], whereas the availability of NO is either still preserved or reduced [5,7,8], presumably depending on the stage of the disease. Furthermore, an improvement in renal vascular NO availability in patients with type 2 diabetes has been documented for treatment with angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers or thiazolidinedione [8,9].

The factors that modulate NO availability in the renal circulation of patients with type 2 diabetes are poorly understood. Gender has repeatedly been shown to affect vascular function in a variety of conditions, but data on the human renal circulation are lacking. In the forearm circulation, stimulated, endothelium-dependent vasodilation is greater in female than in male patients with diabetes [10]. We hypothesized that NO availability in the renal circulation might be greater in female patients with type 2 diabetes, and that this could be the result of reduced levels of renal vascular oxidative stress.

Methods

Patient selection

The patients who were treated in our outpatient clinic for type 2 diabetes or enrolled in our training programme for patients with type 2 diabetes were asked to take part in the present study when they fulfilled the following inclusion criteria: age between 30 and 75 years; HbA1c <9%; casual 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, any current antihypertensive medication and treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers within the previous 3 months, lipid-lowering therapy, insulin therapy, current use of oral contraceptives or oestrogen replacement therapy and active smoking. All patients gave their written informed consent prior to study inclusion. The patients who were treated with an oral hypoglycaemic agent were asked to withhold the morning dose on the day of the clearance study. The study protocol was approved by the Clinical Investigations Ethics Committee of the University of Erlangen-Nürnberg.

Infusion protocol

Systemic haemodynamic parameters (i.e. BP and heart rate) were monitored by an oscillometric device (Dinamap 1846 SX; Criticon, Norderstedt, Germany). Renal haemodynamic parameters were determined by the constant infusion input clearance technique with inulin and sodium $p$-aminohippurate (Clinitalfa, Basel, Switzerland) for glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, as previously reported [5,9,11].

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 was reached, and the additional administration of experimental substances was started. First, $N(G)$-monomethyl-$L$-Arginine (l-NMMA) was administered intravenously (i.v.) as a bolus infusion (3 mg/kg over 5 min) followed by constant infusion (1.25 mg/kg over 25 min) to determine the availability of NO in the renal circulation. Then, l-arginine (l-arginine hydrochloride 6%; University Hospital Pharmacy, Erlangen, Germany) was administered i.v. at a dose of 100 mg/kg over 30 min to reverse NO synthase (NOS) inhibition by excess substrate availability and to restore baseline renal haemodynamic conditions. Over the following 30 min, l-arginine infusion was continued at 100 mg/kg, but vitamin C (45 mg/kg; Cebion® forte; Merck, Darmstadt, Germany) was co-infused over the same time to reduce superoxide levels and, therefore, to determine levels of oxidative stress in the renal circulation. Blood samples to determine inulin and $p$-aminohippurate concentrations were drawn at 0, 120, 150, 180 and 210 min. During the last 5 min of each infusion step, blood pressure was monitored every minute, and the mean of these measurements was given. Renal blood flow was calculated as RPF/(1-haematocrit), renal vascular resistance (RVR) as mean BP/RBF and filtration fraction (FF) as GFR/RPF. All renal haemodynamic 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. Urinary albumin excretion rates (in mg albumin/g creatinine) from first morning urine samples were determined by using a turbidimetric method on a BN ProSpec® analyser (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Urinary creatinine concentration was analysed by using a modified Jaffé method on an AU2700 analyser (Olympus UK Ltd, Watford, UK). Measurements of $p$-aminohippurate and inulin were performed after the completion of the study from the blood samples centrifuged immediately at 4 °C and stored at −21 °C. $p$-Aminohippurate was measured by the method of Smith et al. [12]; inulin was determined indirectly with an enzymatic method following conversion to fructose. Each blood sample was measured in duplicate with a coefficient of variation of <5%.

Statistics

Analyses were performed using SPSS Software (release 15.0; SPSS Inc., Chicago, IL, USA). After confirmation of normal distribution by Kolmogorov–Smirnov tests, unpaired Student’s t-tests were used to compare parametric clinical data, whereas Wilcoxon rank-sum tests were used for nonparametric data. To compare renal haemodynamic responses between the two groups, ANOVA for repeated measurements was used. Data were given as mean ± standard deviation, and a P-value <0.05 (two-sided) was considered statistically significant.

Results

The baseline characteristics of the study cohort are given in Table 1. Genders were well matched with regard to age and most other clinical parameters. Men had a slightly lower body mass index based on greater body surface area, higher diastolic BP, lower HDL cholesterol and higher serum creatinine levels. Importantly, baseline renal haemodynamic parameters indexed to body surface area were similar between the groups.

The changes of systemics haemodynamics during the infusion protocol are given in Table 2. During infusion of l-NMMA, BP increased and heart rate decreased in both groups. The systolic BP increase was more pronounced in females, which was associated with a greater decrease of heart rate, whereas diastolic BP and mean BP increased similarly in both groups. These haemodynamic changes
were reversed during subsequent infusion of l-arginine, and the co-infusion of vitamin C did not lead to any further changes.

The changes in renal haemodynamics are given in Table 3. The infusion of L-NMMA induced a reduction in RPF in both groups, which was greater in female subjects (Figure 1 for percentage changes). The greater availability of NO in the renal circulation in females was also reflected by a greater increase of RVR during the infusion of L-NMMA (Figure 2 for percentage changes). GFR and FF increased during the infusion of L-NMMA, with no difference between the two groups. L-arginine reversed the changes in RPF and RVR to baseline. The subsequent co-infusion of vitamin C increased RPF in both groups, with a greater increase of RPF in male subjects. Again, this was reflected by opposite changes in RVR. GFR and FF were not disparately affected during co-infusion of vitamin C in the two groups.

In a historical cohort of healthy control subjects without diabetes (n = 38 males, n = 36 females), there were no significant differences in the reduction of RPF in response to L-NMMA infusion (-10.1 ± 12.0 versus -9.3 ± 7.9% of baseline RPF, n.s.), nor in response to co-infusion of l-arginine and vitamin C (+10.4 ± 17.6 versus +14.3 ± 13.2% of baseline RPF, n.s.)

**Discussion**

The main finding of our study is that NO availability in the renal circulation is greater in female than in male patients with type 2 diabetes, which is associated with reduced intrarenal levels of oxidative stress in the female patients. To our knowledge, this is the first report on effects of gender on renal endothelial function in patients with type 2 diabetes.
In the human forearm circulation, gender does not affect endothelial function in healthy subjects [13,14]. Similarly, our small cohort of healthy control subjects did not demonstrate any gender-related difference in renal vascular NO availability or oxidative stress. In patients with diabetes, only one study has examined the impact of gender on endothelial function of the forearm vasculature. In that study, gender did not have any effect on L-NMMA-induced vasoconstriction in patients with type 1 diabetes or healthy controls [10]. In contrast, acetylcholine-induced, endothelium-dependent vasodilation was more pronounced in female than in male patients with type 1 diabetes [10]. We are not aware of any investigations addressing this question specifically in humans with type 2 diabetes, nor of any studies on the role of oxidative stress. In the current study, we could clearly demonstrate that in contrast to the data in the forearm circulation, L-NMMA induced vasoconstriction of the renal circulation is greater in female than in male patients with type 2 diabetes, which is associated with reduced levels of oxidative stress in female patients. Whether this is due to differences between vascular beds or due to differences between type 1 and type 2 diabetes has to be studied in the future.

What could be the pathogenetic role of a higher NO availability in the renal circulation of female patients with type 2 diabetes? Recent studies in experimental models of diabetic nephropathy indicate that vascular synthesis of NO protects from progression of renal lesions in diabetes. Wild-type mice, in particular the most commonly used C57BL/6J strain, resist the development of advanced lesions of the kidney, when diabetes is induced by injection of streptozotocin. In sharp contrast, induction of diabetes in mice with deletion of endothelial NO synthase (eNOS) on a C57BL/6J background leads to severe renal lesions, histologically very similar to advanced diabetic lesions of human kidneys [15–17]. Furthermore, elegant experimental evidence indicates that endothelial function of the renal vasculature predicts the progression of renal disease; in the 5/6th nephrectomy model, renal endothelial function of the removed kidney predicts albuminuria and the decline of GFR in the remaining kidney [3]. Severity of endothelial dysfunction, a common finding in patients with diabetes [10,18–20], may therefore be an important accelerating factor for the progression of diabetic nephropathy, and human trials directly addressing this question are eagerly awaited.

In line with this experimental evidence, male gender is an independent risk factor for a more rapid decline of renal function in a variety of renal diseases, including polycystic kidney disease and some types of glomerulonephritis [21–23]. In patients with type 1 diabetes participating in the Diabetes Control and Complications Trial, male gender

**Table 3. Changes of renal haemodynamic parameters in male and female patients with type 2 diabetes during the infusions of L-NMMA, L-arginine and L-arginine plus vitamin C**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infusion</th>
<th>Males (n = 41)</th>
<th>Females (n = 39)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔRPF (ml/min/1.73 m²)</td>
<td>L-NMMA</td>
<td>−60 ± 52*</td>
<td>−89 ± 69*</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>L-arginine</td>
<td>+4 ± 62</td>
<td>+2 ± 87</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>+ Vitamin C</td>
<td>+60 ± 85*</td>
<td>+37 ± 86*</td>
<td>0.05</td>
</tr>
<tr>
<td>ΔRVR (mmHg/ml/min/1.73 m²)</td>
<td>L-NMMA</td>
<td>+3 ± 2*</td>
<td>+5 ± 2*</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>L-arginine</td>
<td>+0 ± 2</td>
<td>−0 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>+ Vitamin C</td>
<td>−3 ± 2*</td>
<td>−1 ± 3*</td>
<td>0.03</td>
</tr>
<tr>
<td>ΔGFR (ml/min/1.73 m²)</td>
<td>L-NMMA</td>
<td>+5 ± 11*</td>
<td>+2 ± 11</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>L-arginine</td>
<td>+10 ± 13*</td>
<td>+7 ± 15*</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>+ Vitamin C</td>
<td>+12 ± 12*</td>
<td>+11 ± 15*</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔFF (%)</td>
<td>L-NMMA</td>
<td>+3 ± 2*</td>
<td>+4 ± 3*</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>L-arginine</td>
<td>+2 ± 2</td>
<td>+1 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>+ Vitamin C</td>
<td>−0 ± 3</td>
<td>+0 ± 3</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to baseline. n.s., non-significant.
was a significant predictor for the progression of albuminuria, independent of age, metabolic control, duration of diabetes and baseline albumin excretion rate [24]. Similar results were reported from a study in normotensive patients with type 1 diabetes in Denmark and from a recent study in 27 805 paediatric and young adolescents patients with type 1 diabetes in Germany [25,26]. In type 2 diabetes, the available data are less supportive of a protective effect of female gender, and in one study, an even more rapid progression of diabetic nephropathy has been demonstrated [27]. However, patients with type 2 diabetes are often characterized by the presence of multiple cardiovascular risk factors including hypercholesterolaemia, which has been shown to impair renal endothelial function [28,29]. The additional presence of hypercholesterolaemia could abolish the higher NO production observed in our relatively healthy female subjects. Future studies therefore need to address the interactive effects of cardiovascular risk factors on renal endothelial function.

By which molecular mechanisms may gender affect renal endothelial function? Studies in rats suggest that gender alters expression and function of eNOS [30,31], and oestrogen specifically has been shown to increase levels of eNOS in the renal medulla of rats [32]. Sex hormones have also been shown to modulate antioxidative capacity of renal tissues and to affect albuminuria and renal damage in spontaneously hypertensive rats [33,34]. Furthermore, lowering testosterone levels by orchietomy has been shown to reduce oxidative stress and renal injury after ischaemia [35]. However, no data in animal models of diabetes nor any data on specific endothelial effects are available. In humans, studies in patients on either hormone replacement therapy or on drugs blocking the actions of oestrogen or testosterone are warranted to further define the role of sex hormones, although one has to be aware of the fact that the underlying conditions may also directly affect endothelial function.

As a limitation of the current study, we did not pharmacologically block autonomic reflexes during infusion of L-NMMA, which has recently been shown to unmask the full contribution of NO to systemic vascular tone [36]. In that study, the acute increase in BP in response to infusion of L-NMMA exceeded 20 mmHg in healthy, normotensive volunteers, despite using a comparatively low dose of L-NMMA [36]. Due to safety reasons, we did not block the autonomous nervous system in our patients with type 2 diabetes, but most likely achieved a more complete blockade of NOS using a higher dose of L-NMMA. In addition, baroreflex regulation of heart rate, cardiac output and sympathetic vasomotor tone seems to be similar in men and women [37], although we are not aware of any specific data in patients with type 2 diabetes. Furthermore, despite the experimental evidence suggesting that the higher NO production in our female patients with type 2 diabetes might be associated with a favourable renal outcome, it is also conceivable that the increased NO production reflects a compensatory response to alterations in other vasoconstrictor or vasodilator mechanisms. As an example, hyperglycaemia, known to activate the renin–angiotensin system, has been shown to cause renal vasoconstriction in female patients, but not in male patients with type 1 diabetes [38]. In addition, the same study showed that angiotensin-converting enzyme inhibition has a greater effect on renal haemodynamics in the female compared to male diabetics [38]. Thus, there seems to be a greater activation of the intrarenal renin–angiotensin system in female patients with diabetes. The higher NO production in the females, as observed in our current study, could therefore be a compensatory mechanism, and thus may not be associated with a better renal outcome. Therefore, long-term follow-up studies are needed to clarify the prognostic impact of specific alterations in renal endothelial function in male and female patients with diabetes.

In conclusion, our data demonstrate that NO availability is greater in female than in male patients with type 2 diabetes that is associated with reduced levels of oxidative stress in females. Given the importance of endothelial function in other vascular beds, and the more recent experimental evidence that renal endothelial function modulates renal injury, future studies are now needed to define the role of the renal endothelium in the initiation and progression of human diabetic nephropathy. In this context, the current study highlights the fact that the modulating role of gender on renal endothelial function deserves greater attention in the future.

Acknowledgements. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 423, TP B5). The authors gratefully acknowledge the expert technical assistance of Ingrid Fleischmann, Dorothea Bader-Schmieder, Simone Pejkovic and Ulrike Heinritz.

Conflict of interest statement. None declared.

References


