Assessment of impaired vascular reactivity in a rat model of diabetic nephropathy: effect of nitric oxide synthesis inhibition on intrarenal diffusion and oxygenation measured by magnetic resonance imaging

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1Institute for Diagnostic and Interventional Radiology, Hannover Medical School, Hannover, Germany; 2Clinic for Nephrology, Hannover Medical School, Hannover, Germany; 3Abbott Laboratories, Hannover, Germany; 4Abbott Products, Hannover, Germany; and 5REBIRTH Hannover, Hannover, Germany

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Hueper K, Hartung D, Gutberlet M, Gueler F, Sann H, Husen B, Wacker F, Reiche D. Assessment of impaired vascular reactivity in a rat model of diabetic nephropathy: effect of nitric oxide synthesis inhibition on intrarenal diffusion and oxygenation measured by magnetic resonance imaging. Am J Physiol Renal Physiol 305: F1428–F1435, 2013. First published September 4, 2013; doi:10.1152/ajprenal.00123.2013.—Diabetes is associated with impaired vascular reactivity and the development of diabetic nephropathy. In a rat model of streptozotocin-induced diabetic nephropathy, the effects of systemic NO synthesis inhibition on intrarenal diffusion and oxygenation were determined by noninvasive magnetic resonance diffusion tensor imaging and blood O2 level-dependent (BOLD) imaging, respectively. Eight weeks after the induction of diabetes, 21 rats [n = 7 rats each in the untreated control group, diabetes mellitus (DM) group, and DM with uninephrectomy (DM UNX) group] were examined by MRI. Diffusion tensor imaging and BOLD sequences were acquired before and after NO synthesis inhibition with N-nitro-L-arginine methyl ester (L-NAME). In the same rats, mean arterial pressure and vascular conductance were determined with and without the influence of L-NAME. In control animals, NO synthesis inhibition was associated with a significant increase of mean arterial pressure of 33.8 ± 4.3 mmHg (P < 0.001) and a decrease of vascular conductance of –17.8 ± 2.0 \mu \text{min}^{-1} \text{100 mmHg}^{-1} (P < 0.001). These changes were attenuated in both DM and DM UNX groups with no significant difference between before and after L-NAME measurements in DM UNX animals. Similarly, L-NAME challenge induced a significant reduction of renal transverse relaxation time (T2*) at MRI in control animals, indicating reduced renal oxygenation after L-NAME injection compared with baseline. DM UNX animals did not show a significant T2* reduction after NO synthesis inhibition in the renal cortex and attenuated T2* reduction in the outer medulla. MRI parameters of tissue diffusion were not affected by L-NAME in all groups. In conclusion, BOLD imaging proved valuable to noninvasively measure renal vascular reactivity upon NO synthesis inhibition in control animals and to detect impaired vascular reactivity in animals with diabetic nephropathy.

diabetic nephropathy; vascular reactivity; blood oxygen level-dependent imaging; diffusion tensor imaging; N-nitro-L-arginine methyl ester; nitric oxide

Diabetes Mellitus (DM) is one of the leading causes of end-stage renal disease (12a, 36a, 59). In the early disease state, it is associated with intrarenal hemodynamic abnormalities including glomerular hyperfiltration (10), an increase in renal plasma flow, and high O2 consumption (1, 18, 27, 58). These factors may contribute to the development and progression to diabetic nephropathy and end-stage renal disease (6, 9, 14, 35, 46, 48). Nitric oxide (NO) mediates vasodilatation, increase of perfusion, and inhibition of O2 consumption in the kidney (5, 24) and is thus associated with increase of renal tissue oxygenation. Dysregulation of NO metabolism seems to be involved in the pathogenesis of diabetes and the progression to diabetic nephropathy (25, 26, 64). N-nitro-L-arginine methyl ester (L-NAME) is an analog of L-arginine and inhibits NO synthesis by NO synthases. Thus, it can be used to investigate alterations of vascular reactivity and renal NO metabolism in hypertension or DM (8, 19, 30, 38, 63).

Functional MRI may be helpful to noninvasively evaluate renal vascular reactivity upon NO synthesis inhibition by measurements of renal tissue oxygenation. Furthermore, historical changes associated with diabetic nephropathy can be examined with MRI (22, 45). In this study, we evaluate a combination of two functional MRI techniques: diffusion tensor imaging (DTI) and blood O2 level-dependent (BOLD) imaging. DTI measures diffusion properties in the tissue, which can be quantified by the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (16). For the calculation of ADC, the magnitude of diffusion along at least three orthogonal directions is averaged, resulting in a global measure of diffusivity in the tissue. As diffusion is not necessarily equal in all directions, measurement of diffusion anisotropy (FA), representing the degree of directed diffusion, provides additional information. We and others (22, 33, 56) have shown that renal fibrosis and diabetic nephropathy are associated with alteration of ADC and FA.

The ability of BOLD imaging to determine tissue oxygenation is based on the fact that the magnetic properties of hemoglobin depend on whether it is present in its oxygenated or deoxygenated form. The transverse relaxation time (T2*) increases with increasing content of oxyhemoglobin (39, 45), so that a decrease of renal T2* may be interpreted as decreased tissue oxygenation due to impaired renal perfusion, decreased blood O2 content, or increased O2 consumption (14, 41, 42). BOLD has been widely used to study intrarenal oxygenation in human and animal studies of diabetes (11, 40, 45, 57), kidney transplantation (17, 54), and renal artery stenosis (7, 47).

The purpose of this study was to determine the effect of systemic NO synthesis inhibition on systemic blood pressure and vascular conductance in a rat model of diabetic nephrop-
athy and to noninvasively investigate the intrarenal effects on diffusion and oxygenation by DTI and BOLD imaging.

METHODS

Animals and induction of diabetes. All efforts were made to minimize both the suffering and numbers of animals. Procedures used in this study were conducted in accordance with the German Animal Welfare Act and with the European Council Directive of November 24th, 1986 (86/609/EEC), and were approved by the responsible governmental agency in Hannover, Germany (AZ 33.9-42502-04-09/1738).

Male Sprague-Dawley rats (6–7 wk, 300 g, Janvier, Le Genest-St-Ise, France) were used for experiments. The following three groups (n = 7 rats each) were examined by MRI: 1) control, 2) DM, and 3) DM with uninephrectomy (DM UNX). At 6–7 wk of age, animals underwent UNX (DM UNX group) or sham operation (control and DM groups) and were fed a high-fat and sucrose diet (DIO362031 Research Diet) afterward. At 12–13 wk of age, DM was induced in DM and DM UNX animals by injection of a low dose of streptozotocin (STZ; 30 mg/kg ip, Zanosar). This protocol induced diabetes in ~90% of the rats. All rats used in the MRI investigation had developed diabetes (serum glucose > 13.9 mmol/l).

Laboratory parameters, blood gas analysis, and renal histology. General parameters, such as body weight and food and water intake, were monitored regularly. Blood was collected from the tail vein within the week of MRI. Blood gas analysis was performed immediately after blood collection into heparinized capillaries using a RAPIDLab 348 analyzer (Siemens Healthcare, Erlangen, Germany). Serum glucose, insulin, and creatinine were determined using a clinical analyzer (Konelab, Thermo Fisher Scientific) and species-specific ELISA kits (EZRMI-13K, Linco). Creatinine clearance and estimated glomerular filtration rate (eGFR) were calculated from serum and urinary creatinine concentrations and urine volume collected over a period of 19 h. Glomerular area was determined from periodic acid-Schiff-stained tissue samples as previously described (22).

Evaluation of the systemic hemodynamic responses to NO synthesis inhibition by L-NAME. Systemic blood pressure was determined noninvasively with a volume pressure recording system and an occlusion tail-cuff device (CODA System, Kent Scientific). For longitudinal experiments, noninvasive blood pressure readings in experimental models are recommended by the American Heart Association (28). The suitability of the CODA System has been previously validated (15).

Animals were trained to the procedure at the start of the study. Throughout the study, every 4 wk, a set of (usually) 15 measurements was taken. Mean results of one set were used to determine mean arterial blood pressure (MAP) and blood flow. Additionally, vascular conductance, defined as flow normalized to MAP (in μL·min⁻¹·100 mmHg⁻¹), was calculated.

Three to four weeks after the MRI investigation, blood pressure measurements were done 30 min after an intraperitoneal bolus injection of L-NAME (10 mg/kg). Results under the influence of L-NAME were compared with those of a previous examination without L-NAME. In preliminary experiments, we verified that the influence of L-NAME was of rapid onset and stable throughout at least 1 h after administration (data not shown).

MRI protocol. Eight weeks after the induction of diabetes, all animals underwent MRI (1.5-T Magnetom Avanto, Siemens Healthcare) using an eight-channel wrist coil. Animals were sedated with midazolam (5 mg/kg ip) followed by an anesthesia with thiopental (30 mg/kg ip). To maintain hydration constant during the examination, physiological saline was infused intraperitoneally at a rate of 2 ml/h. Respiratory motion was reduced by imaging the animals in the supine position with respiratory triggering. Proton density (PD) turbo spin-echo images were acquired in the axial and oblique coronal planes with the following parameters: repetition time (TR)/echo time (TE) = 1,930/41 ms, field of view (FOV) = 100 × 80 mm, matrix = 256 × 204, slice thickness = 2 mm, and number of slices = 20. The coronal plane was oriented along the long axis of the right kidney, and the FOV and matrix were adjusted to obtain an isotropic in-plane resolution. For diffusion measurements, a fat-saturated, single-shot spin-echo echo-planar DTI sequence was applied matching the coronal plane of PD images. The sequence parameters were as follows: TR/TE = 5,600/98 ms, b values = 0 and 300 s/mm², diffusion directions = 6, FOV = 128 × 72 mm, matrix = 128 × 72, number of averages = 9, parallel imaging acceleration factor = 2, slice thickness = 2 mm, number of slices = 10, and acquisition time = 6 min.

Furthermore, BOLD images were acquired in one central coronal plane using a multiecho gradient echo sequence with the following parameters: TR = 184 ms, 12 TE (TE = 6.18–53.04 ms, echo spacing = 4.26 ms), FOV = 100 × 100 mm, matrix = 256 × 256, and number of averages = 10. DTI and BOLD sequences were acquired before and 5 min after an intraperitoneal bolus injection of the NO synthesis inhibitor L-NAME (10 mg/kg ip).

Magnetic resonance data analysis. DTI and BOLD MRI were analyzed by two readers blinded to the animal group assignment on an external workstation using Syngo software (Siemens Healthcare) and OsiriX 3.9.2 (Pixmeo, Geneva, Switzerland). The results represent values of one reader. The results of the second reader were used to determine interobserver variability. Parameter maps of FA and ADC were calculated as previously described (16, 22, 36, 37). FA quantifies diffusion anisotropy in the tissue and is scaled from 0 (no preferred diffusion direction, isotropic diffusion) to 1 (only one diffusion direction, completely anisotropic diffusion). ADC is a quantitative value of global diffusivity in the tissue and was calculated using a monoexponential fit. Furthermore, T2* maps were generated with Siemens software by fitting a single-exponential function to the signal intensity versus TE curve. A decrease in T2* implies a decrease in renal PO2.

On morphological images and parameter maps, the renal cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla were identified (55). Regions of interest with a size of 10–50 pixels each were placed into the four anatomic layers of the kidney on ADC and FA maps as well as T2* maps (Fig. 1). Mean ADC, FA, and T2* values were determined separately for each of the anatomic layers in all animals before and after L-NAME. As T2*

Fig. 1. Placement of regions of interest (ROIs) into the anatomic layers of the kidney. A: histology of the anatomic layers of the rat kidney. CO, cortex; ISOM, outer stripe of the outer medulla; ISOM, inner stripe of the outer medulla; IM, inner medulla. These four layers could be identified on transverse relaxation time (T2*) maps (B) and diffusion images (C), and ROIs were placed as shown. ROIs were copied from b0 images to apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps. Mean T2*, ADC, and FA were calculated separately for all anatomic layers.
values in the inner medulla are highly influenced by the high water content in renal tubules and collecting ducts and have been shown to be unreliable to measure tissue oxygenation (13, 41), we only considered T2* values of the renal cortex and outer medulla for analysis and interpretation.

Statistical analysis. Statistical analysis was performed with Prism Graph 5.0 and SigmaStat. Values are given as means \( \pm \) SE. P values of <0.05 were considered statistically significant. Mean values within the groups (control, DM, and DM UNX) were calculated before and after NO synthesis inhibition for blood pressure, vascular conductance, and MRI parameters (ADC, FA, and T2*). The significance of differences between groups was evaluated by two-way (group and \( \lambda \)-NAME) repeated-measures ANOVA. For other parameters, group differences were analyzed by one-way ANOVA. ANOVAs were followed by post hoc multiple-comparison tests. Coefficients of variation (CVs) were used to evaluate the interobserver variability of MRI measures.

RESULTS

Laboratory parameters and blood gas analysis. STZ-treated animals of both DM and DM UNX groups had significantly increased serum glucose and decreased insulin levels compared with control animals within the week of MRI (\( P < 0.001 \); Table 1). Functional and histological changes of the kidney consistent with diabetic nephropathy were more pronounced in DM UNX animals than in DM animals without UNX, as we have previously described (22). In DM rats, eGFR was decreased and postmortem histology revealed glomerular hyper trophy, especially in DM UNX rats (Table 1). Blood gas analysis did not reveal significant differences in \( \text{PO}_2 \) or \( \text{PCO}_2 \) or differences in hematocrit levels (Table 1).

Evaluation of the systemic hemodynamic responses to NO synthesis inhibition by \( \lambda \)-NAME. MAP at baseline was significantly higher in DM UNX animals compared with control animals (144 \( \pm \) 5 vs. 124 \( \pm \) 3 mmHg, \( P < 0.01 \); Fig. 2). Vascular conductance was lower in DM animals (21.8 \( \pm \) 1.3 \( \mu \text{l} \cdot \text{min}^{-1} \cdot 100 \text{ mmHg}^{-1} \), \( P < 0.05 \)), and there was a trend toward lower values in DM UNX animals (21.3 \( \pm \) 1.2 \( \mu \text{l} \cdot \text{min}^{-1} \cdot 100 \text{ mmHg}^{-1} \), \( P = 0.05 \)) compared with control animals (27.0 \( \pm \) 1.5 \( \mu \text{l} \cdot \text{min}^{-1} \cdot 100 \text{ mmHg}^{-1} \)). NO synthesis inhibition by \( \lambda \)-NAME in control animals induced a significant increase of MAP, from 124 \( \pm \) 3 to 158 \( \pm \) 1 mmHg (\( P < 0.001 \)), and a significant decrease of vascular conductance, from 27.0 \( \pm \) 1.5 to 9.2 \( \pm \) 0.9 \( \mu \text{l} \cdot \text{min}^{-1} \cdot 100 \text{ mmHg}^{-1} \) (\( P < 0.001 \)). However, no significant changes of MAP and vascular conductance were observed in DM UNX rats (Fig. 2).

Intrarenal diffusion by DTI. Baseline FA values of all anatomic layers were significantly reduced in DM UNX animals and inversely correlated with the extent of interstitial fibrosis and tubular and glomerular damage, as previously described (22). However, NO synthesis inhibition and the related changes in MAP and vascular conductance had no effect on diffusion anisotropy in all groups (Fig. 3B). Furthermore, \( \lambda \)-NAME injection was not related to changes of ADC in all groups and all anatomic layers with the exception of the inner medulla in DM animals (Table 2). Representative ADC and FA maps of a control animal and an animal with DM UNX before and after \( \lambda \)-NAME injection are shown in Fig. 4.

CVs for interobserver variability were <5% for ADC of all layers and for FA of inner medulla and inner stripe of the outer

Table 1. Characteristics of the different groups

<table>
<thead>
<tr>
<th>Value</th>
<th>Control Group</th>
<th>DM Group</th>
<th>DM UNX Group</th>
<th>P Value (by ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>728 ( \pm ) 18</td>
<td>598 ( \pm ) 20</td>
<td>568 ( \pm ) 15</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>7.9 ( \pm ) 0.2</td>
<td>25.6 ( \pm ) 0.5</td>
<td>27.6 ( \pm ) 1.2</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>634 ( \pm ) 78</td>
<td>123 ( \pm ) 10</td>
<td>81 ( \pm ) 16</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml/min</td>
<td>2.50 ( \pm ) 0.67</td>
<td>2.08 ( \pm ) 0.25</td>
<td>1.28 ( \pm ) 0.32</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Glomerular area/kidney, ( \times 10^3 ) ( \mu \text{m}^2 )</td>
<td>9.3 ( \pm ) 0.3</td>
<td>9.3 ( \pm ) 0.2</td>
<td>11.2 ( \pm ) 0.3</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ( \pm ) 0.01</td>
<td>7.35 ( \pm ) 0.08</td>
<td>7.34 ( \pm ) 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{PO}_2 ), mmHg</td>
<td>40.8 ( \pm ) 1.0</td>
<td>41.0 ( \pm ) 3.2</td>
<td>40.0 ( \pm ) 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{PCO}_2 ), mmHg</td>
<td>55.6 ( \pm ) 3.0</td>
<td>50.6 ( \pm ) 6.9</td>
<td>52.3 ( \pm ) 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>52.7 ( \pm ) 0.5</td>
<td>54.7 ( \pm ) 0.7</td>
<td>56.4 ( \pm ) 3.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. Rats were divided into the following three groups: control, diabetes mellitus (DM), and DM with uninephrectomy (UNX). Body weight, laboratory parameters, and blood gas analysis were obtained 7–8 wk after the induction of DM within the week of MRI. Study groups were compared using one-way ANOVA, and \( P \) values are shown. NS, not significant.
Intrarenal oxygenation by BOLD MRI. At baseline, T2* values of all anatomic layers were not significantly different between control and DM animals. A significant positive correlation of cortical T2* with eGFR normalized with glomerular area was observed \( r = 0.49, P < 0.05 \). This correlation was even better when only DM animals were considered \( r = 0.68, P < 0.05 \); Fig. 5). NO synthesis inhibition was associated with a significant decrease of T2* values, indicating a decrease of renal oxygenation, in all anatomic layers in control animals and DM animals without UNX. T2* changes were more pronounced in the medulla than in the renal cortex. In the inner stripe of the outer medulla of control animals, T2* decreased from 30 ± 2 to 22 ± 2 ms after L-NAME injection \( (P < 0.001) \) and in DM animals without UNX T2* decreased from 31 ± 2 to 23 ± 1 ms \( (P < 0.001) \). In DM UNX animals, the effect of NO synthesis inhibition was less pronounced (Table 3 and Fig. 3A). Representative T2* maps of a control animal and a DM UNX animal before and after L-NAME injection are shown in Fig. 4.

CVs for interobserver variability of T2* measurement were <5% for all anatomic layers.

**DISCUSSION**

This is, to the best of our knowledge, the first study investigating the systemic and intrarenal effects of NO synthesis inhibition using BOLD and DTI in a rat model of diabetic nephropathy and additional kidney volume reduction. The systemic and intrarenal effects of L-NAME-induced NO synthesis inhibition on MAP and vascular conductance and on tissue oxygenation, respectively, were attenuated in animals with diabetic nephropathy compared with control animals.

By combining a high-fat diet with the application of a low STZ dose, the disease model used for this study closely replicates the characteristics of DM in combination with metabolic syndrome (44, 51). Furthermore, kidney volume reduction in the DM UNX group was associated with an acceleration of the development of diabetic nephropathy (22, 32, 52), so that different stages of the disease could be investigated. Accordingly, renal tubulointerstitial fibrosis and glomerulosclerosis, as typical signs of diabetic nephropathy, were more pronounced in the DM UNX group, as previously described (22).

We determined the systemic effects of L-NAME on blood pressure and vascular conductance simultaneously by tail-cuff measurement and volume pressure recording technology. At

<table>
<thead>
<tr>
<th>ADC, ( \times 10^{-3} ) mm²/s</th>
<th>FA</th>
<th>P Value</th>
<th>Before L-NAME</th>
<th>After L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td>Before L-NAME</td>
<td>After L-NAME</td>
</tr>
<tr>
<td>Control group</td>
<td>2.11 ± 0.12</td>
<td>2.12 ± 0.07</td>
<td>NS</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>DM group</td>
<td>2.39 ± 0.07</td>
<td>2.48 ± 0.04</td>
<td>NS</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>2.54 ± 0.06</td>
<td>2.63 ± 0.05</td>
<td>NS</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>OSOM</td>
<td></td>
<td></td>
<td>Before L-NAME</td>
<td>After L-NAME</td>
</tr>
<tr>
<td>Control group</td>
<td>1.93 ± 0.08</td>
<td>1.89 ± 0.05</td>
<td>NS</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>DM group</td>
<td>2.05 ± 0.08</td>
<td>2.04 ± 0.08</td>
<td>NS</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>2.14 ± 0.07</td>
<td>2.23 ± 0.08</td>
<td>NS</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td><strong>ISOM</strong></td>
<td></td>
<td></td>
<td>Before L-NAME</td>
<td>After L-NAME</td>
</tr>
<tr>
<td>Control group</td>
<td>1.92 ± 0.06</td>
<td>1.90 ± 0.06</td>
<td>NS</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>DM group</td>
<td>2.01 ± 0.10</td>
<td>2.10 ± 0.09</td>
<td>NS</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>2.02 ± 0.07</td>
<td>2.19 ± 0.10</td>
<td>NS</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td><strong>IM</strong></td>
<td></td>
<td></td>
<td>Before L-NAME</td>
<td>After L-NAME</td>
</tr>
<tr>
<td>Control group</td>
<td>1.96 ± 0.08</td>
<td>1.92 ± 0.09</td>
<td>NS</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>DM group</td>
<td>1.89 ± 0.09</td>
<td>2.00 ± 0.07</td>
<td>&lt;0.01</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>2.01 ± 0.07</td>
<td>2.17 ± 0.09</td>
<td>NS</td>
<td>0.46 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. Diffusion properties were measured by diffusion tensor imaging [apparent diffusion coefficient (ADC) and fractional anisotropy (FA)] before and after nitric oxide (NO) synthesis inhibition by N-nitro-L-arginine methyl ester (L-NAME) in the different groups and anatomic layers of the kidney. \( P \) values indicate whether the effect of NO synthesis inhibition was significant (by two-way repeated-measures ANOVA followed by post hoc multiple comparison with the Holm-Sidak method). OSOM, outer stripe of the outer medulla; ISOM, inner stripe of the outer medulla; IM, inner medulla.
baseline, MAP was significantly higher and vascular conductance was lower in DM animals compared with control animals. NO synthesis inhibition was associated with a significant increase of MAP and decrease of vascular conductance in control animals, which is consistent with previous studies (4, 20, 49) and can be explained by reduced NO-mediated vasodilatation after l-NAME injection. In DM animals, the systemic vascular reactivity upon l-NAME injection was attenuated, and no significant changes of MAP and vascular conductance were observed. In contrast, in rat models of DM induced by high STZ doses and without a high-fat diet, the reaction upon l-NAME injection was similar in DM and control rats.

Fig. 4. MRI parameter maps before and after NO synthesis inhibition with l-NAME. Examples show a control animal and DM UNX animal. A and B: MRI parameter maps of T2* (top), representing tissue oxygenation, FA (middle), and ADC (bottom) at baseline and after NO synthesis inhibition with l-NAME of one control animal (A) and one DM UNX animal (B). At baseline, T2* values were not different between control and DM UNX animals, whereas FA was reduced in the DM UNX animal. After l-NAME in this control animal, T2* in the IM was reduced from 56 to 46 ms (A, top), whereas T2* was only reduced from 44 to 39 ms in the DM UNX animal (B, top). ADC and FA values were unchanged after l-NAME in both animals.

Fig. 5. Correlation of cortical T2* and estimated glomerular filtration rate (eGFR). Shown are the significant correlations of cortical renal T2* with eGFR normalized with glomerular area in all animals (A) and in DM animals only (B).
and, in addition, baseline blood pressure was not different (38, 53). This discrepancy may be due to differences in the animal models with respect to the amount of pancreatic β-cells that were destroyed by different doses of STZ injection, comorbidity with metabolic syndrome, and the severity of histological changes of diabetic nephropathy. Furthermore, other factors, such as strain, sex, and age of the study animals, may influence the pathophysiology in different studies.

The intrarenal effects of L-NAME on diffusion anisotropy and on tissue oxygenation could be measured by DTI and BOLD imaging, respectively. As previously shown, at baseline, diffusion anisotropy (FA) in DM animals with UNX was significantly reduced and FA negatively correlated with the degree of diabetic nephropathy (22). However, NO synthesis inhibition by L-NAME had only minimal effects on diffusion properties in the rat kidney in all groups. This may be explained by the fact that DTI was acquired with only two b values of 0 and 300 s/mm², so that differentiation of microperfusion and pure diffusion by intravoxel incoherent motion (IVIM) imaging and biexponential models was not feasible.

The suspected effect of L-NAME on renal microperfusion might be detectable by IVIM imaging. For example, Heusch et al. (21) and Wittsack et al. (60) have shown, using ECG- and respiratory-gated DTI and diffusion sequences with multiple b values, that ADC and FA vary during the cardiac cycle and argued that these changes are due to changes in renal perfusion. However, Wittsack et al. also demonstrated that pure diffusion was not influenced by changes in renal perfusion. Thus, we suspect that, with the imaging protocol used for the present study, FA mainly represents pure diffusion anisotropy and, to a lesser extent, microperfusion and tubular flow. IVIM imaging with biexponential models to separately quantify pure diffusion and microperfusion might help to detect L-NAME effects also by diffusion weighted imaging.

Renal tissue oxygenation was determined by BOLD imaging. Using a free-breathing sequence with multiple image averages, good image quality without visible artifacts was achieved. However, inhomogeneities of the static magnetic field caused by respiratory motion may not be excluded. When interpreting BOLD images, it is important to note that T2* values may not only represent tissue oxygenation but may also be influenced by tissue water content, blood volume, and renal perfusion. Furthermore, it has been shown that renal vascular oxygenation and tissue oxygenation may change independently from each other (23, 29). As, in particular, T2* values in the inner medulla have been shown to be highly influenced by other factors than tissue oxygenation, such as a higher water content (13, 41), similar to most other BOLD studies, only T2* values of the renal cortex and outer medulla were considered for analysis and interpretation (11, 30, 43). In contrast to renal diffusion anisotropy, T2* values at baseline were not significantly different between DM and control animals. Current literature on baseline T2* values in diabetes is highly controversial both in human and animal studies, with some studies reporting increased baseline T2* values in diabetes (45, 62) and others reporting decreased T2* (57) or no change of T2* at baseline (12). These discrepancies may be due to differences between animal models, differences in the stage of diabetes, and differences in glomerular filtrations between studies. Therefore, BOLD imaging in combination with a challenge (e.g., L-NAME or water load) might be more meaningful and reproducible to detect renal pathology than baseline BOLD measurements.

In our study, cortical T2* values significantly correlated with eGFR normalized with body weight and glomerular area. The correlation was even better when only DM animals were considered. This finding is in contrast to the recent publication of Michaely et al. (34), who found no significant correlation of renal T2* values with eGFR. The difference in our study may be explained by the fact that we examined a specific disease model of experimental diabetic nephropathy, whereas Michaely et al. (34) determined renal T2* values in a large nonspecific patient population. Although baseline T2* values were not different between groups of DM and control animals in our study, the combination of BOLD imaging with L-NAME challenge evidenced significant group differences. T2* values in control animals significantly decreased after L-NAME injection in all anatomic layers, indicating decrease of tissue oxygenation. In DM animals with UNX, the effect of NO synthesis inhibition on tissue oxygenation was attenuated, interpreted as reduced renal vascular reactivity in diabetic nephropathy. The attenuation of renal vascular reactivity was accompanied by reduced systemic vascular reactivity in DM UNX animals. As NO increases renal Po2 by mediating vasodilatation, increase of perfusion, and inhibition of O2 consumption in the kidney (5, 24), the opposite effect with decrease of renal Po2 and T2* can be expected by NO synthesis inhibition with L-NAME in normal rats. The observed reaction upon L-NAME injection in the control group is also consistent with Li et al. (31), who investigated changes of tissue oxygenation upon NO synthesis inhibition by BOLD imaging in rats and validated the results by invasive P02 measurements. The reduction of vascular reactivity in DM UNX animals may be explained by lower NO bioavailability in DM, which is caused by oxidative stress and interaction of NO with ROS (50, 61). The lower baseline NO availability in DM animals may be a reason for the diminished effects of L-NAME to decrease NO synthesis and subsequently to reduce tissue oxygenation. Corresponding to our results in DM animals with UNX, Palm et al. (38), who used invasive measurements of renal O2 tension, found a

<table>
<thead>
<tr>
<th>T2*, ms</th>
<th>Before L-NAME</th>
<th>After L-NAME</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>26 ± 1</td>
<td>24 ± 1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DM group</td>
<td>31 ± 1</td>
<td>28 ± 1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>32 ± 2</td>
<td>31 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>OSOM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>23 ± 1</td>
<td>19 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM group</td>
<td>26 ± 1</td>
<td>21 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>26 ± 1</td>
<td>22 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ISOM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>30 ± 2</td>
<td>22 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM group</td>
<td>31 ± 2</td>
<td>23 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM UNX group</td>
<td>29 ± 2</td>
<td>24 ± 2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. Transverse relaxation time (T2*) values were measured by blood O2 level-dependent before and after NO synthesis inhibition by L-NAME in the different groups and anatomic layers of the kidney. P values indicate whether the effect of NO synthesis inhibition was significant (two-way repeated-measures ANOVA followed by post hoc multiple comparison with the Holm-Sidak method).

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significant reduction of renal $\text{PO}_2$ upon l-NAME injection in control rats, whereas in STZ-induced diabetic rats this response was reduced.

A limitation of the present study is the fact that we did not validate BOLD MRI by invasive measurement of tissue oxygenation. However, Li et al. (31) observed good overall agreement between changes of the BOLD signal and invasive measurement of $\text{PO}_2$ after injection of l-NAME in normal rats. Therefore, we assume that changes of the BOLD signal upon l-NAME injection in the renal cortex and outer medulla in this study are largely attributed to changes in renal tissue oxygenation, although changes of blood volume or renal perfusion may have an additional effect on T2*. Furthermore, we only investigated the effects of l-NAME on intrarenal oxygenation and diffusivity at one selected time point. Monitoring changes of renal vascular reactivity and renal pathology in the same animal over time is intended for future studies.

In conclusion, BOLD and DTI represent promising non-invasive imaging techniques for the examination of alterations of renal vascular reactivity and structure in diabetic nephropathy in animal and human studies. Renal diffusion properties are stable upon NO synthesis inhibition and reflect histopathological changes of diabetic nephropathy. BOLD imaging combined with l-NAME challenge detects differences in renal vascular reactivity upon NO synthesis inhibition between control animals and DM animals with UNX by measurement of intrarenal oxygenation.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


