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Early Changes With Diabetes in Renal Medullary Hemodynamics as Evaluated by Fiberoptic Probes and BOLD Magnetic Resonance Imaging

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Abstract

Objective—We sought to evaluate the influence of streptozotocin (STZ)-induced diabetes on renal outer medullary pO₂ and blood flow by invasive microprobes and to demonstrate feasibility that blood oxygenation level-dependent (BOLD) magnetic resonance imaging (MRI) can monitor these changes.

Materials and Methods—A total of 60 Wistar-Furth rats were used. Diabetes was induced by STZ in 48. Animals were divided into OxyLite group (n = 30) and BOLD MRI groups (n = 30) each with a 5 subgroups of 6 animals: control and 2, 5, 14, and 28 days after induction of diabetes. Outer renal medullary oxygen tension and blood flow were measured by the combined OxyLite/OxyFlo probes.

Results—Both OxyLite and BOLD MRI showed a significant increase in the renal hypoxia levels after STZ at all time points. However, no changes were observed in the outer renal medullary oxygen tension and blood flow between diabetic and control groups.

Conclusions—These preliminary results suggest that hypoxic changes can be detected as early as 2 days in rat kidneys with diabetes by BOLD MRI and that these early changes are not dependent on blood flow.

Keywords

kidney; diabetes; oxygenation; BOLD MRI; OxyLite

Diabetes mellitus is a leading cause of end-stage renal disease (ESRD). The pathogenesis of diabetic nephropathy is not yet well understood.¹ At the onset of diabetes mellitus, the intrarenal hemodynamic abnormalities manifest in the form of glomerular hyperfiltration² and high oxygen consumption³ and may be the factors that are responsible for the onset and progression of diabetic nephropathy (DN).⁴ Several mechanisms associated with hypoxia may be involved in the progression of nephropathy in patients with diabetes.^{5,6}

Although the significance of renal hypoxia is well established in ischemic nephropathy, the same is not appreciated in diabetic nephropathy. With molecular markers being identified to be useful in monitoring hypoxia,⁷ there is new interest in studying renal hypoxia in diabetes.^{8–10} At least 2 major causes of renal hypoxia are appreciated within diabetic kidneys, one as

the result of increased oxygen consumption to support sodium reabsorption during the hyperfiltration stage (functional hypoxia) and the other as the result of oxidative stress (imbalance of vasoactive substances), both of which are relevant to pathophysiology of DN.¹¹ The relative contribution of these 2 mechanisms may be variable over time. It is suspected that functional hypoxia is the dominant factor early in the progression and oxidative stress may become dominant in the later stages.

The maintenance of normal renal blood perfusion and oxygenation is critical in the control of renal function. The oxygen tension (pO_2) in the renal medulla is low because of countercurrent oxygen diffusion between descending and ascending vasa recta. In addition, the high rate of ion transport activity in the thick ascending limbs of the loops of Henle also contributes to a low pO_2 in the renal medulla. Therefore, the renal medulla is especially susceptible to further decrease in the pO_2 .¹²

It has been postulated that hypoxia may ultimately cause glomerular damage, leading to a progressive loss of renal function.¹³ Furthermore, complete renal ischemia in diabetic rats has been shown to cause accelerated renal injury with lesions similar to those seen in human diabetic nephropathy.¹⁴ A recent study has shown that the pO_2 in chronic diabetic rats is decreased throughout the renal parenchyma.¹⁵ Moreover, the outer medullary cells of diabetic rats have been shown to exhibit a significantly higher consumption of oxygen when compared with cortical cells.¹⁵ Hypoxia also has been associated with the development of diabetic nephropathy despite controlled glycemia.⁶

Measurements of tissue oxygenation have been performed primarily in animal models using microelectrodes, a labor intensive and extremely fragile technique and hence limited to a handful of laboratories around the world. The availability of alternate and more robust technologies could allow for more widespread use of these measurements. Oxy-Lite is a novel laser-based technology that is not as fragile and labor intensive compared with microelectrode measurements. Blood oxygenation level-dependent (BOLD) magnetic resonance imaging (MRI) has been shown to be very useful in monitoring intrarenal oxygenation.^{16–32} A major advantage of the noninvasive BOLD MRI is the ability to perform similar measurement in humans.

The primary objective of this study was to demonstrate the feasibility of monitoring early changes in renal oxygenation by BOLD MRI and to correlate them with those measured by novel laser based invasive pO_2 and blood flow probes. This is important for proper interpretation of BOLD MRI data because they do not differentiate changes as the result of oxygen supply versus demand. The secondary objective was to determine whether there is any temporal variation in the level of hypoxia during a 4-week period after the administration of STZ.

MATERIALS AND METHODS

Animals

Experiments were performed on 60 inbred male Wistar Furth rats weighing between 220 and 320 g purchased from Harlan Laboratories (Madison, WI). The rats were housed at the institutional animal care facility that is approved by the American Association for the Accreditation of Laboratory Animal Care. The rats had free access to food and water throughout the study. All protocols were approved by the Institutional Animal Care and Use Committee.

The animals were age matched and divided into 2 groups, OxyLite and BOLD MRI groups. Each group has 30 rats. The groups were then subdivided into 5 subgroups (6 rats in each group): control, 2, 5, 14, and 28 days after induction of diabetes groups. Animals from each of

the 5 subgroups were subjected to measurements of outer renal medullary oxygen tension, blood flow by the combined OxyLite/OxyFlo probes (Oxford Optronics, Oxford, UK), or BOLD MRI. Mean arterial pressure (MAP) was monitored in OxyLite/OxyFlo group.

Diabetes Induction

Diabetes was induced under a light anesthesia, Isoflurane (Abbott Laboratories, North Chicago, IL), by an injection of streptozotocin (Sigma Chemical Co., St. Louis, MO; STZ 50–55 mg/kg body weight dissolved in saline 1 mL/kg of saline) via the lateral tail vein.³³ Control rats received an equal volume of vehicle. Animals with blood glucose levels of 15 mmol/L or less at 24 hours after STZ administration were excluded from the study.

Protocol 1: Evaluation of Outer Medullary Blood Flow (OMBF) and Oxygen Tension (pO₂) in Diabetic Rats

The animals were anesthetized with ketamine (60–100 mg/kg intramuscularly; Abbott Laboratories, North Chicago, IL) and inactin (100 mg/kg intraperitoneally; Sigma Chemical Co., St. Louis, MO). Catheters were inserted in the right femoral artery and vein for monitoring blood pressure and administering intravenous infusions, respectively. The rats received an intravenous infusion of a 0.9% NaCl solution containing 2% albumin at a rate of 500 μ L/h/100 g per body weight for control rats and double the rate for diabetic rats throughout the experiment because diabetic rats have a higher loss of fluid. The left kidney was exposed by an abdominal incision and immobilized in a stainless-steel cup. The kidney was surrounded by cotton wool soaked in saline and mineral oil. The animals were allowed a 30-minute equilibration period. Throughout the experiment, the body and kidney temperatures were monitored. Microprobes were inserted into the kidney at 3 to 4 mm depth to reach the renal outer medulla using a micromanipulator.

The OxyLite measures pO₂ by determining the O₂-dependent fluorescent lifetime of ruthenium chloride that is immobilized at the tip of a fiber-optic probe.³⁴ OxyLite has been previously used to measure pO₂ in tumor,³⁵ spleen³⁴ and, more recently, in the kidney.³⁶ The OxyFlo uses laser-Doppler flowmetry for continuous measurements of tissue blood perfusion in arbitrary blood perfusion units. The technique is most facile in monitoring changes in blood flow rather than absolute blood flow rates.

The probes used in this study were combined probes with a tip size ~450 μ m (OxyLite with temperature probe ~350 μ m + OxyFlo ~ 100 μ m). The probes are calibrated at the manufacturer before shipment; the calibration for each probe was scanned into the computer by using a barcode wand. The pO₂ and blood flow signals from the probes were a 5-second average value and were recorded to disk with the use of a data-acquisition system (Powerlab, Chart v5 for Windows; AD Instruments, Colorado Springs, CO).

At the end of each experiment, animals were euthanized by an overdose of sodium pentobarbital (Sleep Away, Fort Dodge Animal Health, IA). Positioning of the probes was confirmed at the end of the experiments by dissection.

Protocol 2: Evaluation of BOLD MRI Signal in the OM of Diabetic Rats

Anesthetized rats (ketamine (60–100 mg/kg intramuscularly; Abbott Laboratories, North Chicago, IL) and Inactin (100 mg/kg intraperitoneally; Sigma Chemical Co., St. Louis, MO) were placed inside the extremity coil within the scanner. To minimize the artifacts produced by respiratory motion, multiple acquisitions (10) were acquired and averaged. Imaging was performed on a 3-T Twin Speed scanner (General Electric Medical Systems, Milwaukee, WI) using a multiple gradient recalled echo (mGRE) sequence without spatial spectral RF pulse (TR/TE/Flip angle/bandwidth/field of view/matrix size = 70/4.4–57.8 milliseconds/30/41.7

kHz/10cm/256 × 256) to acquire 16 T_2^* -weighted images. The choice of parameters was made to maintain the minimum SNR to be above 2 necessary to avoid errors in R_2^* estimation as the result of noise at higher echo times.³⁷ The slice thickness of the images was 2 mm and the in-plane resolution was 0.4 mm × 0.4 mm. The image acquisition time was 3 minutes. The rate of spin dephasing $R_2^*(=1/T_2^*)$, which is closely related to the content of deoxyhemoglobin in blood was used as a BOLD parameter. A decrease in R_2^* implies an increase in tissue pO_2 and vice versa. R_2^* maps were constructed using FUNCTOOL by fitting a single exponential function to the signal intensity versus echo time data. Regions of interest were placed in both medulla and cortex (CO) on the R_2^* map using anatomic image as a reference.

Statistical Evaluation

Comparisons between different groups were performed using analysis of variance followed by the Dunnett and Tukey posttest (Prizm 4, GraphPad Software, Inc, San Diego, CA). $P \leq 0.05$ was considered statistically significant.

RESULTS

Effects of STZ-Induced Diabetes on Kidney Weight, Blood Glucose, and MAP in the OxyLite Group

The average body weight, kidney weight, blood glucose, and MAP measured in each subgroup are summarized in Table 1. Because of attrition, only 5 animals were available for 28-day group. The body weight showed a decrease in the mean value after the induction of diabetes but reached statistical significance only for time points 2 and 28 days when compared with the age-matched control group. Blood glucose was significantly elevated in all STZ-treated rats (>400 mg/dL) compared with the age-matched control group. Kidney weight was found to be greater in the diabetic rats but did not reach statistical significance for any group compared with control rats. Mean arterial pressure measurements showed no appreciable difference between the groups.

Effect of STZ-Induced Diabetes on Renal pO_2 and OMBF in the OxyLite Group

Figure 1 summarizes the renal oxygenation in the outer medulla (OM) as measured with OxyLite. The control group had an average outer medullary pO_2 of 36 ± 1.4 mm Hg and outer medullary blood flow of 500 ± 19 blood perfusion units (arbitrary blood perfusion units). Induction of diabetes produced a significant decrease in renal pO_2 of 20% at 2 days and up to 59% at 28 days (Fig. 1A). No changes were observed in the OMBF between diabetic and control groups (Fig. 1B) at any of the time points.

Effect of STZ-Induced Diabetes on the BOLD MRI Signal in BOLD Group

Anatomic images and corresponding R_2^* maps from one representative animal in each of the experimental subgroups are shown in Figure 2. The brightness in medulla increases with each group, indicating a progressive decrease in the oxygenation in renal medulla. Although the images are suggestive of increased R_2^* values in the cortex, considering the relatively limited spatial resolution this may be at least in part due to partial volume effects. Changes along the cortico-medullary junction region in which the tissue appears more heterogenous postdiabetes, possibly suggesting changes in oxygenation along the medullary rays and vasa recta are also apparent.

Figure 3 summarizes the R_2^* values measured in the cortex and outer medulla in the different experimental groups. Due to attrition, only 4 animals were available for the 28 day group. Oxygenation determined by BOLD signal was found to be higher in the cortex (32 ± 0.6

seconds⁻¹) than in OM (41 ± 1.5 seconds⁻¹) in control rats. There was a significant decrease in the renal oxygenation as shown by the higher value in the cortical and medullary R_2^* in diabetic rats compared with controls over time. The increase in the BOLD signal was progressive with the highest increase observed with the 28-day group for both CO and OM.

DISCUSSION

In the present study, we have demonstrated that renal oxygenation as determined by both OxyLite and BOLD MRI decreases significantly during early phases of diabetes and that it is not dependent on changes in regional blood flow. These are consistent with recent reports.^{15, 27}

Diabetes was induced by a single dose of STZ, which causes hyperglycemia by destroying the β cells in the pancreas^{38,39} and is widely used as an experimental model of type I diabetes in different species, such as cats, rats, and mice. Although potential nondiabetic effects of STZ cannot be completely ruled out, other studies have shown that type 1 diabetes is the predominant feature of rats treated with STZ at the dose used in the present study.⁴⁰

It has been previously reported that STZ increases blood glucose levels, decreases body weight, and has a mortality rate around 20%.⁴¹ In our study, we observed a mortality rate of approximately 18%. It is possible that the mortality rate is related to the effects of hyperglycemia on the cardiac function causing arrhythmia.⁴² Blood glucose levels were elevated approximately 3.5-fold at 24 hours after the intravenous administration of STZ. We also observed that diabetic rats did not gain weight compared with the control group, with a difference of up to 20% in the body weight. Even though kidney weight was increased in diabetic rats, the values did not reach statistical significance at any of the time points. This may be partly attributable to the wide distribution of body weights at baseline. There was no change in the MAP during the period that diabetes was evaluated. There is evidence that nitric oxide production may be increased in diabetes, at least in the early stages, and may contribute to maintaining the MAP at normal levels.^{43,44}

The relatively large size of the OxyLite probe ($\sim 350 \mu\text{m}$) compared with Clark electrodes ($\sim 10 \mu\text{m}$) leads to a concern regarding possible interference with regional micro-circulation. However, the agreement between the findings in this study with previous reports using microelectrodes at 3 mm depth^{15,45,46} does suggest that reliable numbers could be obtained using OxyLite in the renal medulla. Also, in a recent report, O'Connor et al have shown that the measurements made with OxyLite in the renal medulla were comparable to those obtained with Clark electrodes.³⁶ In addition, in the same work the authors point out that cortical measurements made at 1 mm depth were less reliable with OxyLite, probably because of insufficient penetration. On the basis of our similar experience with the cortical measurements, we did not measure cortical pO_2 with OxyLite in this study.

In all 4 diabetic groups (2, 5, 14, and 28 days) the pO_2 was considerably lower compared with control rats. These changes cannot be attributed to blood flow changes as illustrated in Figure 1. Diabetes is known to increase the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ (sodium pump).¹⁵ Furthermore, a previous study has shown that renal tubules from diabetic rats consumed around 40% more oxygen than tubules from non-diabetic rats. This increased oxygen consumption was thought to be mainly related to high metabolic activity.⁴⁷ Palm et al¹⁵ also demonstrated that oxygen consumption of renal medullary and cortical cells were higher in diabetic rats. The fact that oxygen consumption is increased in the diabetic kidney, predominantly in the medulla, because of an increase in metabolic activity is likely to contribute to the enhanced hypoxia seen in diabetes. Palm et al¹⁵ proposed such chronic oxygen deprivation, especially in the renal medulla could contribute to the progression of renal disease in diabetes.

Our BOLD results showed the cortex in control animals has considerably lower R_2^* than OM consistent with previous reports showing higher tissue pO_2 in the cortex.⁴⁸ Additionally, after inducing diabetes, the BOLD signal significantly and progressively increased in both CO and OM, consistent with our observed responses measured using OxyLite. The measurements in the cortex by BOLD MRI should be viewed with caution owing to the limited spatial resolution. It is also important to point out that the inner medulla on the R_2^* map is relatively dark even though it has been shown previously based on microelectrode measurements that the tissue pO_2 may be the lowest in this region.^{15,45} This has been a consistent finding to date,^{20,21,23,26,27} and we do not yet know the exact cause. It might result from high fluid content in inner medulla. We also speculate that this may be related to the relatively low blood volume and/or hematocrit within this region. Because the technique is inherently dependent on the presence of hemoglobin, lower blood volume and/or hematocrit will affect the relative sensitivity. This emphasizes the fact that although changes in oxygenation in tissue can be reflected as changes in the regional R_2^* , R_2^* measured in different regions does not necessarily correlate with regional differences in tissue oxygenation. Also, a recent article demonstrated the feasibility of correcting for bulk susceptibility effects on R_2^* measurements,⁴⁹ which may prove beneficial for BOLD MRI measurements. BOLD MRI is ideally suited for studying outer medullary oxygenation status and in the cortex when it becomes hypoxic. BOLD MRI signal is determined by the deoxyhemoglobin content, and as such can be influenced by both blood supply and oxygen consumption. By comparing the BOLD MRI data with those obtained with invasive probes used to measure pO_2 and blood flow, it is clear that the observed changes are primarily related to changes in oxygen extraction rather than blood flow.

We have not measured glomerular filtration rate in this study. Data from the literature reveal that hyperfiltration phenomenon can be seen as early as 2 days after induction of diabetes.⁵⁰ Ries et al²⁷ suggested that changes in medullary oxygenation observed by BOLD MRI at 5 days post induction were related to hyperfiltration.

These preliminary results support the future use of BOLD MRI to identify early changes in renal hemodynamics in patients with diabetes, thereby allowing for noninvasive, objective monitoring of effects of novel interventions designed to avoid development of vascular complications such as diabetic nephropathy. The noninvasive nature of the technique potentially allows for following longitudinal changes in the same animal. It is also feasible to combine perfusion measurements by MRI.⁵¹ Because OxyLite/Oxyflo probes are MRI compatible, it is possible to perform these measurements simultaneously with BOLD MRI. However, some logistical hurdles (eg, MRI compatible probe holders) need to be overcome. Also, because BOLD MRI is a noninvasive procedure, we potentially could have obtained the longitudinal data in the same animal. Alternately, we could have performed BOLD MRI followed by fiber-optic probe measurements in each animal. However, in this preliminary study, we did not attempt these to keep the logistics for animal handling relatively simple. The exact cause for the observed early changes in hypoxia is not yet clear. It is possible that hyperglycemia itself could result in enhanced hypoxia because of osmotic diuresis resulting in enhanced reabsorptive work.⁹

In conclusion, our data demonstrate that BOLD MRI can monitor early changes in hypoxia within diabetic kidneys and these changes are in agreement with OxyLite measurements. These observed early changes cannot be attributed to changes in blood flow. Future studies are warranted to understand the cause of these early changes and how they may be reversed.

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FIGURE 1.

Effect of induced diabetes on the renal oxygenation and blood flow in rats (control, n = 6; 2 days after inducing, n = 6; 5 days, n = 6; 14 days, n = 6; 28 days, n = 5). A, The outer medulla pO₂ was measured with OxyLite. B, The outer medulla blood flow was measured with OxyFlo. Error bars show the standard error over different animals.

FIGURE 2.

Effect of diabetes on the BOLD MR images. R_2^* maps from one representative rat from each group: control, 2, 5, 14, and 28 days. Note the brightness increase on the R_2^* maps with time after STZ administration, suggesting increasing levels of hypoxia. Also apparent are differences in the relative texture in the parenchyma, which may suggest increased heterogeneity after the induction of diabetes, especially in the cortico-medullary region, possibly reflecting changes in oxygenation status along the medullary rays and vasa recta.

FIGURE 3.

Effect of diabetes on (A) the renal R_2^* signal in the cortex and (B) outer stripe of outer medulla (OSOM). Note the progressive increase in R_2^* values (suggesting increasing levels of hypoxia) in both cortex and medulla during the 4-week period. Error bars show the standard error over different animals. Nondiabetic (n = 6), 2 days (n = 6), 5 days (n = 6), 14 days (n = 6), and 28 days (n = 4) after induction of diabetes.

Effect of STZ-Induced Diabetes on Body Weight, Kidney Weight, Blood Glucose Levels, and MAP in Wistar Furth Rats in the OxyLite Group

TABLE 1

	Control (n = 6)	2 Days (n = 6)	5 Days (n = 6)	14 Days (n = 6)	28 Days (n = 5)
Body weight (g)	303 ± 17	248 ± 13*	260 ± 9	259 ± 12	231 ± 15*
Kidney weight (g)	1.02 ± 0.04	1.10 ± 0.05	1.15 ± 0.06	1.21 ± 0.01	1.12 ± 0.03
Blood glucose (mg/dL)	127 ± 8	425 ± 27*	408 ± 26*	410 ± 23*	441 ± 31*
MAP (mm Hg)	95 ± 4	99 ± 4	100 ± 4	100 ± 5	88 ± 5

Date are expressed as means ± SEM.

* $P < 0.05$ by analysis of variance compared with controls.