

## Evaluation of intra-renal oxygenation in db/db mice by BOLD MRI

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### INTRODUCTION

Diabetes promotes chronic renal hypoxia, which is a pathway leading to end stage renal disease (ESRD) [J Am Soc Nephrol 17: 17-25, 2006]. Among the many techniques available to evaluate intra-renal oxygenation, blood oxygen level-dependent (BOLD) MR imaging is currently the only non-invasive method that can be readily used *in vivo* in both animal models and humans [Nephron Clin Pract 2006; 103: c58-65]. BOLD MRI studies in streptozotocin (STZ)-induced rat model of Type I diabetes have shown increased levels of medullary hypoxia at very early time points following STZ administration [Invest Radiol 2007; 42: 157-162; J Magn Reson Imaging 2003; 17: 104-13]. A commonly used mouse model to investigate diabetic kidney disease is the db/db mouse [Am J Physiol Renal Physiol 2003; 284: F1138-44]. These transgenic mice are homozygous for the db gene and show signs of diabetes as early as 4-6 weeks of age [Am J Pathol 1972; 66: 193-224]. Their littermates (db/m), on the other hand, are completely normal. These mice have a mutation in the LepR gene, which leads to obesity and diabetes [Am J Physiol Regul Integr Comp Physiol 2000; 278: R320-330]. The progression of diabetes in mice mirrors that in humans [Am J Physiol Renal Physiol 2003; 284: F1138-1144; Am J Pathol 2005; 167: 327-336]. In this study, we used BOLD MRI technique to evaluate renal medullary oxygenation levels in db/db mice compared to their littermates db/m mice. Based on our previous findings in the Type I diabetes rat model, we hypothesized that db/db mice will show enhanced medullary hypoxia compared to their control db/m mice.

### MATERIAL AND METHODS

Experiments were performed on seven inbred male C57BLKS/J (db/db) mice (46.7±1.1 gm; Jackson Laboratory, Bar Harbor, ME, USA) and six of their healthy littermates (db/m) (26.3±1.5 gm) at age 10 weeks old. Six of the db/db mice (50.5±2.2 gm) were rescanned at 15 weeks. The study protocol was approved by the institutional animal care and use committee. The animals were anesthetized with ketamine (100 mg/kg *ip*; Abbott Laboratories, North Chicago, IL, USA). Their glucose levels were checked using a One Touch Ultra glucometer (LifeScan, Mountain View, CA).

Imaging was performed on a 3.0T Twin Speed whole body scanner (General Electric Medical Systems, Milwaukee, WI, USA) using a custom-designed surface coil [J. Magn. Reson. Imaging 2007; 25:635-638] and a multiple gradient-recalled echo (mGRE) sequence (TR/TE/flip angle/bandwidth=101.5 msec/6.3-3.2 msec/30°/31.25 kHz) to acquire six T2\* weighted images. The field of view was 4 cm, with a matrix size of 256 by 256. The mice were placed in a supine position above the coil with four paws taped to the pad. Given the size of the coil, only one kidney was routinely covered. Based on scout images, one coronal slice was selected in the middle of the kidney for BOLD MRI data acquisition. The signal intensity *vs.* TE data were fit to a single decaying exponential function to determine the value of R2\* (= 1/T2\*), which was used as a semi-quantitative measure of relative tissue oxygenation. An increase in R2\* indicates a decrease in tissue pO2. R2\* maps were generated from the T2\*-weighted images using the Funtool feature on the Advanced Workstation 4.0 (GE, Milwaukee, WI, USA). Regions of interest (two to four per region) were drawn over the renal cortex and outer medulla. The mean and standard deviation of R2\* in the two renal regions were recorded.

### RESULTS

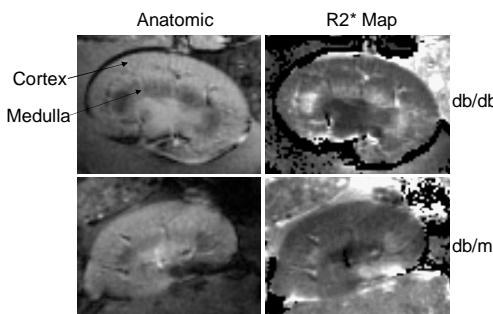


Figure shows representative examples of BOLD MRI data obtained in a diabetic db/db and a control db/m mouse. The brightness on the R2\* map reflects the relative oxygenation status of the kidney. The outer medulla of the diabetic kidney is significantly brighter than that of the healthy kidney, indicating a decreased tissue oxygenation. This is consistent with our previous findings in the Type I diabetes model [Invest Radiol 2007; 42: 157-162]. Table summarizes the R2\* measurements in medulla (MR2\*) and cortex (CR2\*) in db/db and db/m at 10 weeks and 15 weeks.

### CONCLUSION AND DISCUSSION

This study for the first time shows evidence of increased renal hypoxia in mouse models of type II diabetes. These observations are consistent with previous reports on type I diabetes model in rats [Invest Radiol 2007; 42: 157-162; JMRI 2003; 17: 104-113]. The db/db mice in our care exhibited polyuria, were significantly larger than their healthy littermates, and had significantly higher blood glucose levels. With BOLD MR imaging, we observed that at ten weeks the renal medullary oxygenation

level was already significantly decreased in diabetic mice. Studies at 15 weeks of age show a further increase in MR2\* and CR2\* values. Our previous report on STZ induced type I diabetes [Invest Radiol 2007; 42: 157-162] suggested very little change in blood flow implying that the observed decrease in oxygenation is predominantly related to increased oxygen consumption.

In conclusion, the results presented here further support the previous observations that kidneys in diabetic subjects display increased hypoxic levels. Further studies are necessary to evaluate the temporal variations and effects of interventions that potentially slow or stop the progression of chronic kidney disease. The key advantage of the BOLD MRI method is that these observations could be translated to human kidneys.

**ACKNOWLEDGEMENT** This work was supported in part by a grant from the National Institutes of Health, RO1-DK073973. We thank Scot Lindsay, Ph.D. (GE Healthcare, Waukesha, WI) for providing the surface coil.

<sup>§</sup> p < 0.05 compared to db/m; <sup>#</sup> p < 0.05 compared to 10 weeks

Species	10 weeks			15 weeks			
	Weight (grams)	BGL (gm/dL)	MR2* (s <sup>-1</sup> )	CR2* (s <sup>-1</sup> )	Weight (grams)	MR2* (s <sup>-1</sup> )	CR2* (s <sup>-1</sup> )
db/db	46.6 ± 1.1	> 600	43.1 ± 5.1 <sup>§</sup>	37.1 ± 3.1 <sup>§</sup>	50.5 ± 2.2	51.7 ± 3.0 <sup>#</sup>	43.1 ± 4.4 <sup>#</sup>
db/m	26.3 ± 1.5	175 ± 38	32.3 ± 3.7	27.1 ± 4.1	-	-	-