Recent studies suggest that renal tissue PO2 is significantly lower in diabetic rats [Diabetologia. 2003;46:1153-1160], and that hypoxia of the kidney plays a major role in the development of acute [N Engl J Med. 1995 Mar 9;332(10):647-55] and chronic renal failure [Clin Exp Pharmacol Physiol. 2006 Oct;33(10):989-96]. BOLD MRI measurements in type 1 diabetic rats (following administration of streptozotocin (STZ)) had documented increased levels of R2* as early as two days following STZ [Invest Radiol 2007 (42):157-162]. Hypothesizing that this may be related to direct effects of hyperglycemia, BOLD MRI measurements were obtained in healthy rats before and following i.v. administration of glucose [Proc. Intl. Soc. Mag. Reson. Med. 16 (2008): 2692]. While modest but statistically significant increases in blood glucose levels and R2* were observed, the magnitude of change was not comparable to those in diabetic rats. It is known that in both healthy animals and humans, glucose stimulates insulin release in order to maintain blood glucose level (BGL) [J. of Clinical Endocrinology and metabolism. 2008 76 (3): 752-756]. In order to achieve high elevations in BGL, the insulin release has to be inhibited with agents such as octreotide. In this study, we pretreated the rats with octreotide prior to initiation of hyperglycemia.

MATERIAL AND METHODS
The study protocol was approved by the Institutional Animal Care and Use Committee. Eight male rats (371±16 grams, seven Sprague-Dawley, one Wistar Furth, Harlan Laboratories, Madison, WI USA) were anesthetized using Inactin (100 mg/kg i.p., St. Louis, MO, USA). The femoral vein was catheterized for administering of glucose and insulin inhibitor octreotide. Imaging was performed on a 3.0T scanner (CVi, GE, Milwaukee, WI, USA) using a multiple gradient recalled echo sequence (TR/TE/dip angle/bandwidth/FOV/slice thickness/NEX = 95ms/2.9-36.5ms /10cm/2mm/12) to acquire eight T2*-weighted images. The in-plane spatial resolution is 0.39mm. The rat kidney was positioned in the middle of the standard knee coil. One transverse slice was selected in the middle of the kidney.

Fresh solutions of glucose (20%) and octreotide (500µg / ml) were prepared on the day of the experiments. Octreotide (Sigma, Louis, MO, USA) 400 µg was administered as a bolus [J.of Gastroenterology and Hepatology, 22 (2007): 1872-1876]. Glucose solution was then administered starting as a bolus (0.6ml) followed by a 90’ continuous infusion [J of Surgical Research, 61, 449-453 (1996)] via a infusion pump (Genie Plus, Kent Scientific, Litchfield, CT, USA). Further sets of T2*-weighted images were obtained every 3 minutes and BGL was monitored every 20’ minutes.

The signal intensity vs. echo time data were fit to a single decaying exponential function to generate R2* map. ROIs were chosen on the maps to obtain values for the mean and standard deviation of R2* in the renal medulla and cortex. The averaged readings of medullary R2* (MR2*), cortical R2* (CR2*) and BGL during period where BGL values reach a plateau were used as post data. The statistical significance of the differences between pre- and post-glucose R2* values was assessed using the two-tailed paired Student’s t-test.

RESULTS
The preliminary results presented here demonstrate that intra-renal oxygenation decrease following acute hyperglycemia as indicated by BOLD MRI measurements. Octreotide assisted in achieving a sustained and higher level of hyperglycemia during glucose administration. Compared with STZ model at 2 day time point [Invest Radiol 2007 (42):157-162], we observed similar levels of hyperglycemia and increase in R2*. The steady state blood glucose level was 391.9±43.7 mg/dL in this study, which is comparable to the STZ model (~400 mg/dL); the post MR2* increased 9.3±1.4 (1/s) which is also comparable to the increase of 11.5±1.6 (1/s) in STZ model. It is now clear that the overproduction of reactive oxygen species (ROS) in diabetes is a direct consequence of hyperglycemia [Diab./Metab. Res. Rev. 17 (2001) 189-212] and that various types of cells including endothelial, vascular smooth muscle, mesangial, and tubular epithelial cells are capable of producing ROS under hyperglycemic condition [Diabetes Res Clin Pract. 2008 Oct 7]. It was also shown previously that treatment with antioxidants improves the oxygenation status in STZ treated rats [Diabetologia. 2003;46:1153-1160]. We have previously shown that NOS inhibition results in increased R2* [J. Magn. Reson. Imaging 2003;17:671-675] and antioxidant such as tempol improves renal oxygenation in hypertensive rats [J. Magn. Reson. Imaging 2005;21:245-248].

In conclusion, administration of glucose with pretreatment with octreotide results in an immediate change in renal oxygenation status as detected by BOLD MRI. The results are consistent with previous observations in STZ model and may explain the very early changes observed in that model. These observations in general further support the role for strict glycemic control in diabetics. Future studies with antioxidant treatment in this model may further verify the contribution of oxidative stress associated with hyperglycemia.

ACKNOWLEDGEMENT
This work was supported in part by a grant from the National Institutes of Health, ROI-DK073973.