Hypoxia accelerated metabolic alterations in the diabetic kidney assessed with hyperpolarized MRS

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Introduction: In diabetic patients, reduced renal oxygen availability is associated with a higher risk of diabetic nephropathy (DN) [1]. Recently, dynamic nuclear polarization hyperpolarized [1-^{13}C]pyruvate has shown an increased lactate conversion (lactate dehydrogenase activity) in the diabetic kidney, as a consequence of increased nicotinamide adenine dinucleotide (NADH) substrate generated by glucose-mediated elevated flux through the polyol pathway, while the pyruvate dehydrogenase activity (PDH) is unaltered (denoted pseudo hypoxia) [2]. In this study, we evaluated the renal oxygenation level and energy metabolism in diabetes-suffering animals in response to altered oxygen availability, using blood oxygen level dependent (BOLD) MRI and hyperpolarized [1-^{13}C]pyruvate MRS, in response to hypoxic, normoxic and hyperoxic conditions. Specifically, we investigated the enzymatic activity of lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and pyruvate dehydrogenase (PDH).

Materials and Methods: Type-1 streptozotocin-induced diabetic rats and control rats were subjected to three levels of inspired oxygen (10%, 21% and 100%) in order to alter blood oxygen content. At each level, oxygenation and metabolism were measured using BOLD MRI and hyperpolarized [1-^{13}C]pyruvate-based MRS. The rats were anesthetized and a tail vein catheter was inserted for injection of hyperpolarized [1-^{13}C]pyruvate. Temperature and respiration were monitored throughout the experiment. Each animal received three injections of 1 mL hyperpolarized [1-^{13}C]pyruvate over 10 s with a 45 min separation. The experiments were performed in a 4.7 T small-bore MR system equipped with Agilent Direct Drive console with Vnmrj 2.3A (Agilent Technologies, Santa Clara, CA). The rat was placed in a ^{13}C/^{1}H volume coil for transmission and a 4-channel array ^{13}C-coil (receive-only) was placed over the kidneys (RAPID Biomedical GmbH, Germany).

Results and discussion: BOLD MRI confirmed that reduced inspired oxygen limits oxygen availability in both diabetic and control kidneys. Reduced oxygen availability in the diabetic kidney altered the energy metabolism of [1-^{13}C]pyruvate by increasing the lactate 23% and 34% alanine formation from pyruvate despite intact oxidative phosphorylation. Interestingly, we found a correlation between the metabolites and interplay with oxygen availability, where the statistically significant correlation between alanine and lactate or bicarbonate. We found a statistically significant difference across the different oxygen levels between the diabetic and control kidney, during hypoxia the alanine and bicarbonate are statistically while the alanine and lactate correlation was indifferent between the control and diabetic kidneys. Our findings revealed increased oxygen sensitivity in the diabetic kidney.

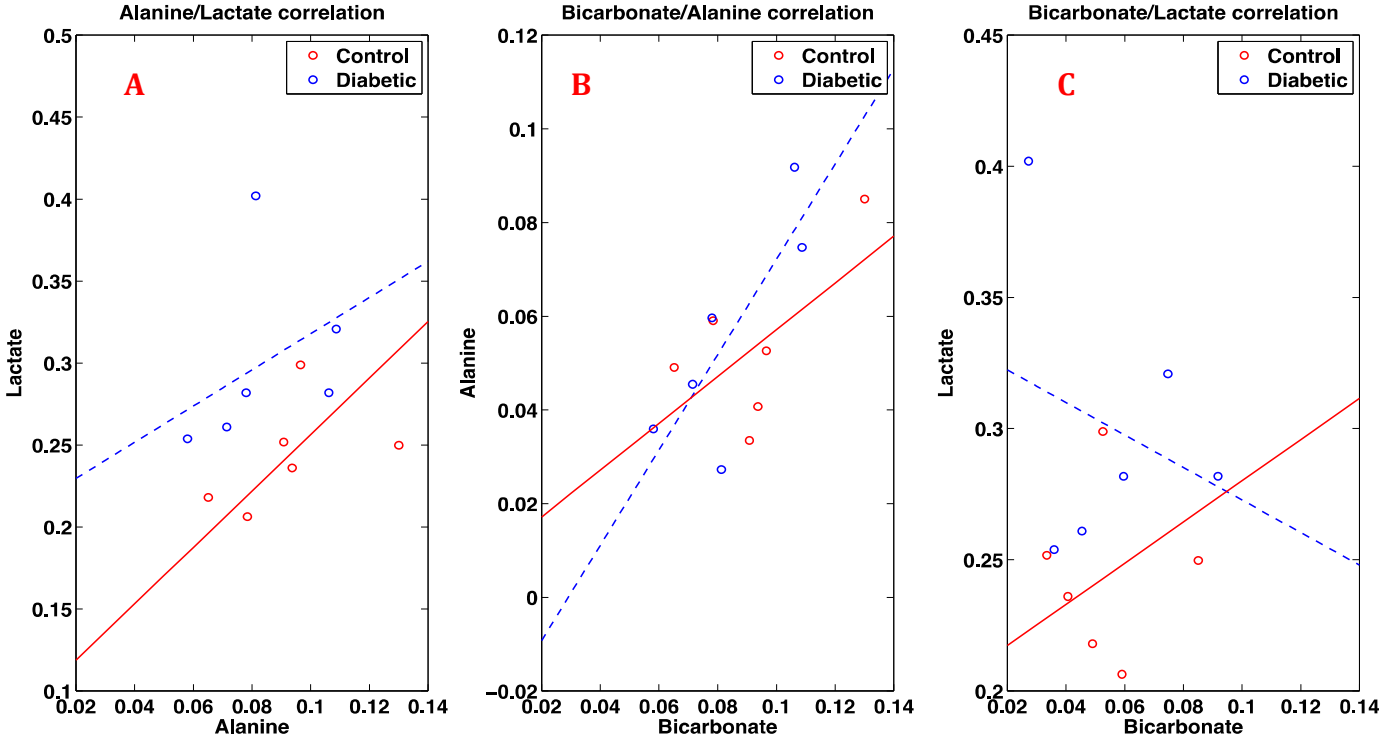

Figure 1: During hypoxic (10% Oxygen) condition the intra metabolite correlations between (A) Lactate and alanine, showing similar (p = 0.53, 95%-CI:-2.5,1.3) (B) Alanine and bicarbonate correlation showing significant correlation and significant difference between the control and diabetic kidney (p = 0.01, 95%-CI: 0.2,0.9). (C) Showing lactate and bicarbonate to be uncorrelated and similar (p=0.21, 95%-CI3.6,0.8),

Conclusion: We find interestingly that the bicarbonate/alanine correlation to be more sensitive than the alanine/lactate correlation, indicating that both alanine and bicarbonate levels are important in the development of diabetic nephropathy and the combined information from ALT and PDH is an important pathophysiological biomarker associated with diabetic nephropathy and oxygen availability.

References: 1. Hochman ME, et al. *Kidney Int* 2007; **71** : 931-938. 2. Laustsen C, et al. *Diabetes Metab Res Rev*. 2013;**29**:125-129.