**METABOLIC DRIVERS OF DICARBONYL STRESS INDUCED BY HIGH GLUCOSE CONCENTRATION IN HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS IN CULTURE**

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**ABSTRACT**

**Background and aim:** Dysfunction of renal proximal tubular epithelial cells (PTECs) in hyperglycemia in diabetes is associated with dysfunction linked to the development and progression of diabetic kidney disease. This is characterised by increased production of transforming growth factor-β leading to tubulointerstitial fibrosis, abnormal salt and glucose transport and increased cell senescence. Glyoxalase 1 (Glo1) catalyses the metabolism of the reactive glycating metabolite, methylglyoxal (MG). Overexpression of Glo1 prevented the development of experimental diabetic kidney disease in mice, even when limited to PTECs and vascular endothelial cells. It therefore appears that dicarbonyl stress induced by hyperglycemia in PTECs may be influential in development of diabetic kidney disease. In this study we sought to investigate the metabolic drivers of increased MG, precursor of major increased advanced glycation endproducts (AGEs) in diabetic kidney disease, by studying human PTECs in primary culture in low and high glucose concentrations.

**Materials and methods:** Human PTECs were incubated in primary culture with 7 mM (model normoglycemia) or 25 mM glucose (model hyperglycemia) for 4 days in quadruplicate, changing medium after 2 days. Glucose consumption and flux of formation of L-lactate and D-lactate were measured by enzymatic assays over the initial 2 days of culture; the latter is a measure of flux of MG formation. The activities of Glo1 and glyoxalase 2 (Glo2) were assayed by spectrophotometric assay after 4 days.

**Results:** When human PTECs were incubated with high glucose concentration *in vitro* for 4 days there was a decrease of Glo1 activity, with respect to low glucose control: 350 ± 19 versus 249 ± 19 mU/mg protein; - 29%, P<0.01. Glo2 activity was unchanged: 39.3 ± 12.3 versus 39.3 ± 2.2 mU/mg protein. There was a 3-fold increase in glucose consumption by PTECs in high glucose cultures: glucose consumption (nmol/day/106 cells) – 18.2 ± 1.4 versus 5.7 ± 1.0; P<0.001. This was associated with an increase in flux of formation of D-lactate – 2.70 ± 0.12 versus 2.21 ± 0.32; + 22%, P<0.05 – and increase in net flux of formation of L-lactate - 293 ± 12 versus 260 ± 0.12; + 13%, P<0.05.

**Conclusion:** Human PTECs suffer down regulation of Glo1 activity and increased in flux of MG formation in model hyperglycemia in primary culture. These are likely metabolic drivers synergising to increase the concentration of MG in PTECs in diabetes. Overexpression or Glo1 inducer therapeutics may correct this and prevent and alleviate diabetic kidney disease.

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