**Distinguishing the Metabolic and Osmotic Effects of Raised Glucose in Human Proximal Tubule Cells**

**Background:** Diabetic Nephropathy is the leading cause of kidney failure. Chronic exposure to high levels of sugar is an important factor in the initiation and development of diabetic Nephropathy (DN). Approximately 90% of glucose filtered by the glomerulus is reabsorbed by the low affinity Na+/glucose co-transporter SGLT2, predominantly expressed in the S1and S2 segments of the proximal tubule. There has been a renewal of interest in SGLT2 since it has become a major target for anti-diabetic drugs. Here we show that under high glucose conditions, in the presence of transforming growth factor beta 1 (TGFβ1), primary human Proximal Tubule Epithelial Cells (PTEC) display pro-fibrotic effects whilst maintaining their epithelial phenotype.

**Methods:** Primary and transformed human PTEC were grown on plain and collagen IV coated culture dishes. Upon reaching 80% confluence, they were treated with D Glucose at either 5mM (normoglycaemia/control), 25mM(hyperglycaemia) or 5 mM D-Glucose + 20 mM L-Glucose (osmotic control) in the presence and absence of TGFβ1, 0.75ng/ml. After 24 hours, cells were lysed. Heparin bead precipitation was applied to the media to isolate heparin-binding proteins. Western blotting was performed with antibodies against SGLT2, SGLT1, the hinge region of Connective Tissue Growth Factor (CTGF) protein and extracellular signal-regulated kinase 1/2 (phospho-Erk1/2).

**Results:** Results from western blotting experiments indicate that primary human PTEC express SGLT2 protein, unlike the transformed cell line HKC8, in which only SGLT1 protein was detected. Treatment with the pro-fibrotic growth factor TGFβ1 did not change SGLT2 protein under control conditions, but decreased SGLT2 significantly during hyperosmolarity (hyperglycaemia and osmotic control). Furthermore, full length secreted CTGF protein (36 & 38 kDa) was significantly increased under raised glucose + TGFβ1 treatment. These significant changes were observed exclusively on the primary PTEC grown on collagen IV. TGFβ1 treatment of primary PTEC cultured on collagen IV for 5 minutes resulted in a dose dependent reduction in cellular phosphor Erk1/2. This was not observed in cells grown on uncoated cultureware.

**Discussion:** The data shows that a “high” D glucose concentration in combination with TGFβ1 is able to exert both an osmotic and metabolic effect on primary human PTEC. High osmotic pressure revealed a TGFβ1-mediated loss of SGLT2. This decrease of SGLT2 is supported by another study1 that showed TGFβ1 signalling downregulated the plasma membrane scaffolding protein Caveolin-1 (Cav-1) in lung myofibroblast cells and reports that osmotic pressure can also alter Cav-1 lipid raft localisation. SGLT2 is associated with Cav-12, thus factors that reduce Cav-1 abundance are likely to reduce SGLT2 expression at the cell membrane. The metabolic effect of raised D glucose caused a significant TGFβ1- induced increase of CTGF, a pro-fibrotic outcome. TGFβ1 alone can induce CTGF expression, as reported by us and others3. However, the concentration of TGFβ1 used in the experiments here was below the threshold required for CTGF induction in human PTEC in culture.

From our data we suggest that the SGLT2 inhibitor class of drugs will not block all the effects of raised glucose on human PTEC. This important finding needs to be considered in future studies in this field. We will now proceed to investigate whether glucose-mediated pro-fibrotic effects described above are regulated by SGLT2 using drugs that selectively block this transporter.

1Sanders et al (2015), *PloS one*, 10(2), p.e0116995

2Lee et al (2012), *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1823(4), pp.971-982

3Phanish et al (2005), *Nephron Experimental Nephrology*, 100(4), e156-e165