

**Background:** Altered connexin expression &/or function is linked to the development and progression of secondary microvascular complications associated with diabetes. Despite this, we know little for the role of these small membrane proteins in the diabetic kidney. This study examines if glucose-evoked changes in TGF- $\beta$ 1 modulate connexin expression and gap junction-mediated intercellular communication in diabetic nephropathy.

**Methods:** Biopsy material was isolated from patients with diabetic nephropathy and stained for connexin-26 and connexin-43. Changes in expression, were corroborated by immunoblot analysis in model epithelial cells from human renal proximal tubules (HK2) cultured in either low glucose (5mmol/L) +/- TGF- $\beta$ 1 (2-10ng/mL) or high glucose (25mmol/L) for 7days. ELISA was used measured TGF- $\beta$ 1 secretion and paired-patch electrophysiology recorded junctional conductance in control versus TGF- $\beta$ 1 treated (10ng/mL) HK2 cells.

**Results:** Connexin-26 expression was significantly up regulated in biopsy material from patients with diabetic nephropathy, compared to normal control (102700 $\pm$ 6226 versus 21030 $\pm$ 4727; n=5,  $P$ <0.01). Similarly, connexin-43 expression increased to 116300 $\pm$ 5908 as compared to control 21460 $\pm$ 10920 (n=5,  $P$ <0.01). In response to high glucose (25mmol/L) treatment for 7days, HK2 cells increased TGF $\beta$ 1 secretion to 994.4 $\pm$ 43.6pg/ml compared to 5mmol/L glucose (334 $\pm$ 14.9pg/ml; n=3;  $P$ <0.01). Immunoblot analysis confirmed that TGF $\beta$ 1 (10ng/mL) up-regulates expression of connexin-26 and connexin-43 to 203.9 $\pm$ 7.5% and 151.1 $\pm$ 7.1% respectively compared to control (n = 4;  $P$ <0.001). Whole cell paired-patch electrophysiology was used to determine the junctional conductance between coupled HK2 cells  $\pm$  TGF- $\beta$ 1 (10ng/ml). TGF- $\beta$ 1 produced decreased junctional conductance to 0.42 $\pm$ 0.2nS compared to control 4.5 $\pm$ 1.3nS (n=5;  $P$ =<0.05).

**Conclusion:** Expression of connexin-26 and connexin-43 increased in biopsy material isolated from patients with diabetic nephropathy, changes corroborated in HK2 cells treated chronically with TGF- $\beta$ 1. Despite this gain in expression, gap junction mediated intercellular conductance was reduced, a feature linked to increased hemi-channel activity.