Diabetic Complications Consortium

Application Title: Kidney energetics ion the development of nephropathy in type 1 diabetes

Principal Investigator: Josephine Forbes

1. Project Accomplishments:

This award has been extended due to unavoidable delays in completing the compliance paperwork. However, as evidenced below we have made substantial progress on the project and aims to have it completed within the time granted for the extension.

2. Specific Aims:

Aim 1: Obtain kidney images of fuel (lactate/fatty acids; 1H-MR) and energy storage content (ATP; 31P-MR) from young T1D individuals (15-18 yrs) with and without evidence of early kidney dysfunction and match these to urinary metabolites and urinary cell energetics.

(i) *Clinical Study*. We have completed the recruitment of and sample collection (plasma/urine/data/PBMCs) from all of the subjects in the clinical cohort. The subjects (n=100) have been divided into their baseline ACR tertiles. The unadjusted baseline data are summarised below:

	Lower Tertile (≤0.66)	Middle Tertile (0.67-1.16)	Upper Tertile (≥1.17)
N	33	33	34
Sex (n Male, %)	(21, 63.6%)	(16, 48.5%)	(17, 50%)
Age (yr)	21 (17.5-22.5)	20 (17.5-22.0)	19 (17-23.25)
Age at diagnosis (yr)	11 (7-14.5)	10 (4-12.5)	9.5 (5.5-12.25)
Diabetes duration (yr)	9.879 ± 4.904	11.42 ± 4.944	10.65 ± 5.762
HbA_1c (%)	8.0 (7.3-8.55)	8.2 (7.45-8.7)	8.45 (7.975-9.1) *
HbA_1c (mmol/mol)	63.94 (56.29-69.95)	66.13 (57.93-71.59)	68.86 (63.67-75.96) *
Fed BG (mmol/L)	11.65 (7.225-14.88)	12 (7.925-15.20)	12.55 (8.2-15.08)
Height (m)	1.75 (1.665-1.795)	1.73 (1.675-1.785)	1.71 (1.65-1.82)
Weight (kg)	80 (69-87)	74 (68.2-78.5)	74.5 (65.85-84.65)
BMI (kg/m^2)	26 (23-29.5)	24 (22.5-26.5)	24.5 (20.75-28.25)
Mean ACR (mg/mmol)	0.5 (0.415-0.57)	0.82 (0.73-0.93) *	1.73 (1.483-4.425) *†
Ur Albumin (mmol/L)	6.1 (5-10.53)	7 (5-10.9)	12.45 (5-47.38)
Ur Cr (mmol/L)	15.04 (10.56-53.50)	22.41 (10.44-56.00)	14.63 (5.095-54.75)
Plasma Cystatin C (ng/ml)	694.8 (619.7-796.6)	615.6 (585.1-754.8)	634.0 (570.0-703.5)*

Ur KIM-1 (ng/mg Cr)	48.84 (17.66-83.22)	28.40 (10.75-83.10)	31.29 (8.307-105.3)
CKD-EPI eGFR	134.7 ± 9.492	136.3 ± 8.115	137.8 ± 11.35
Schwartz eGFR	119 (104-130.5)	117 (107.5-124.5)	108 (97.5-121.8)

Table 1: Data are expressed as Mean+/-SD or Median (IQR)*P<0.05 vs Lower tertile; † P<0.05 vs Middle tertile.

(ii) *Urinary metabolomics*: Assessment and quantification of central carbon metabolites and purine nucleotide concentrations in urine have been completed. We are just waiting for the lipidomic analyses to be completed.

(iii) *Imaging:* A new coil had been installed on the 7T Magnetom and we are just in the final stages of testing the kidney imaging. We have had almost all the patients recruited consent to MRI and so we will be able to randomly select the smaller cohort with equal numbers from the upper and lower/middle tertiles of urinary ACR. We anticipate that we will have at least one quarter of the patient cohort imaging completed before the end of 2016.

(iv) *Metabolic flux analyses in cells*: We have obtained accurate information using flow cytometry for the urinary cell types. Unfortunately these exfoliated urinary cells had lost their capacity for oxidative phosphorylation and so we have substituted measurement of the patient PBMCs instead to assess metabolic fuel flexibility in these patients. The data show that there is a change in the metabolic behaviour of the PBMCs taken from patients represented in the upper tertile of ACR, ie those most at risk of future nephropathy. These cells show a reduced capacity for energetic changes under stress.

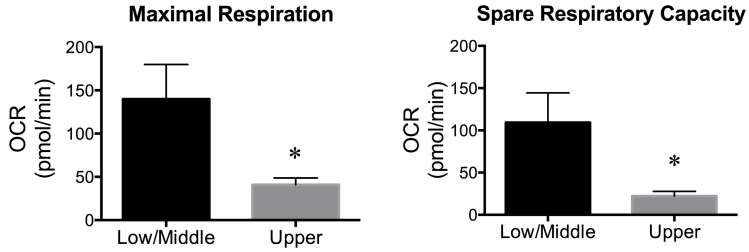

Figure 1: Seahorse XF24 analyses of PBMCs during metabolic stress testing in the presence of glucose and glutamate. OCR – oxygen consumption rate.

*P<0.05 vs lower/middle tertile (n=45/20)

Aim 2: Use human kidney cells (proximal tubular cells and podocytes) derived from nephrectomy to better understand mitochondrial energy generation in type 1 diabetes.

We have been culturing cells form the nephrectomies obtained to date and the collection on actual numbers is approximately as projected. We are waiting for sufficient numbers in each group since we wish to perform the analyses as a group.

3. Publications and Presentations:

1. Australian Diabetes Society 2016. Invited Keynote Speaker - Prof Josephine Forbes. Diabetic nephropathy – An energetic crisis? Gold Coast, Australia.

2. International Society of Nephrology Forefronts 2016. Diabetic nephropathy – An energetic crisis? - Prof Josephine Forbes. San Diego, USA.
3. Mater Translation of Research into Clinical Practice symposia 2016. Prof Josephine Forbes. Diabetic nephropathy – An energetic crisis? Brisbane Australia.