Histological and Molecular Assessment of Adipose Tissue Health and its Correlation with Glomerular Damage in the Zucker Diabetic Sprague Dawley (ZDSD) Rat Model of Diabetic Kidney Disease

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BACKGROUND

- Pathological changes in adipose tissue (AT) arising during the progression of type 2 diabetes mellitus (T2DM) have been implicated in end-organ damage in organs such as the kidneys.
- Adiponectin is an adipocytokine involved in a putative adipose-renal axis. A mediating factor is the expression of adiponectin receptors on glomerular podocytes. Adiponectin receptor knockout mice displayed kidney damage similar to early diabetic kidney disease (DKD)[1].

AIMS

- To characterize AT morphological change and adiponectin gene expression in T2DM in ZDSD rat model
- To assess whether changes in these parameters correlated with structural changes in the glomerulus.

METHODS

- 32 ZDSD rats were humanely sacrificed at 3 timepoints, 26, 32 and 38 weeks of age.
- Prior to euthanasia, a 16-hour urine collection was performed for each animal and using a colourimetric assay albumin excretion rate were calculated.
- Epididymal visceral AT necroscopy samples were obtained and processed for histological staining. Adipocyte area was measured using ImageJ.
- Necropsy samples of renal cortex were obtained from 11 animals and processed for transmission electron microscopy (TEM).
- Glomerular basement membrane (GBM) thickness and podocyte foot process frequency and diameter were measured using ImageJ.

RESULTS

- 15 animals remained normoglycaemic (blood glucose < 7 mmol/L), 10 animals progressed to prediabetes (7 – 11 mmol/L) and 8 animals progressed to diabetes (> 11 mmol/L) (Table 1).
- Adipocyte diameter and adiponectin gene expression were decreased in diabetes (Fig. 1 (b), (c))
- Albumin excretion (µg/hr) was increased in the diabetes (Fig. 1 (d))
- Podocyte foot process frequency and diameter were unchanged by blood glucose, but GBM thickness was increased in prediabetes (Fig. 2 (c)) and corelated with blood glucose (Spearman r: 0.8, p: 0.003).
- GBM thickness correlated with AT adiponectin gene expression (Fig. 2 (d))

RESULTS

Table 1: Biochemical and ultrastructural results from 11 ZDSD rats

Animal ID	Blood glucose (mmol/L)	Albumin (µg/h)	Podocyte foot process frequency	Podocyte foot process diameter (nm)	GBM thickness (nm)
1	5.67	152.23	17.50	377.86	212.84
2	5.80	56.59	16.33	431.97	229.25
3	6.47	121.96	18.33	443.31	214.14
4	6.80	119.80	14.33	494.96	242.74
5	6.97	135.60	16.33	397.32	256.25
6	7.17	81.62	15.50	387.60	248.10
7	7.50	368.63	15.67	445.06	292.06
8	8.23	922.20	18.00	497.45	320.22
9	18.57	621.35	18.33	378.75	250.76
10	26.57	1,781.53	13.50	509.08	267.27
11	33.33	3,853.07	16.00	467.89	269.09

Table 1: Blood glucose, albuminuria and ultrastructural results. Blood glucose results are an average of 3 measurements taken over the 3 weeks prior to euthanasia. Blood glucose measurements had an upper limit of 33.3 mmol/L. The lowest values of albumin, foot process diameter, foot process frequency and GBM thickness are portrayed in green, progressing through yellow and to red for highest values. Podocyte foot process frequency measurements indicate average number of foot processes per 8 µm GBM.

Figure 1: AT measures and urine albumin

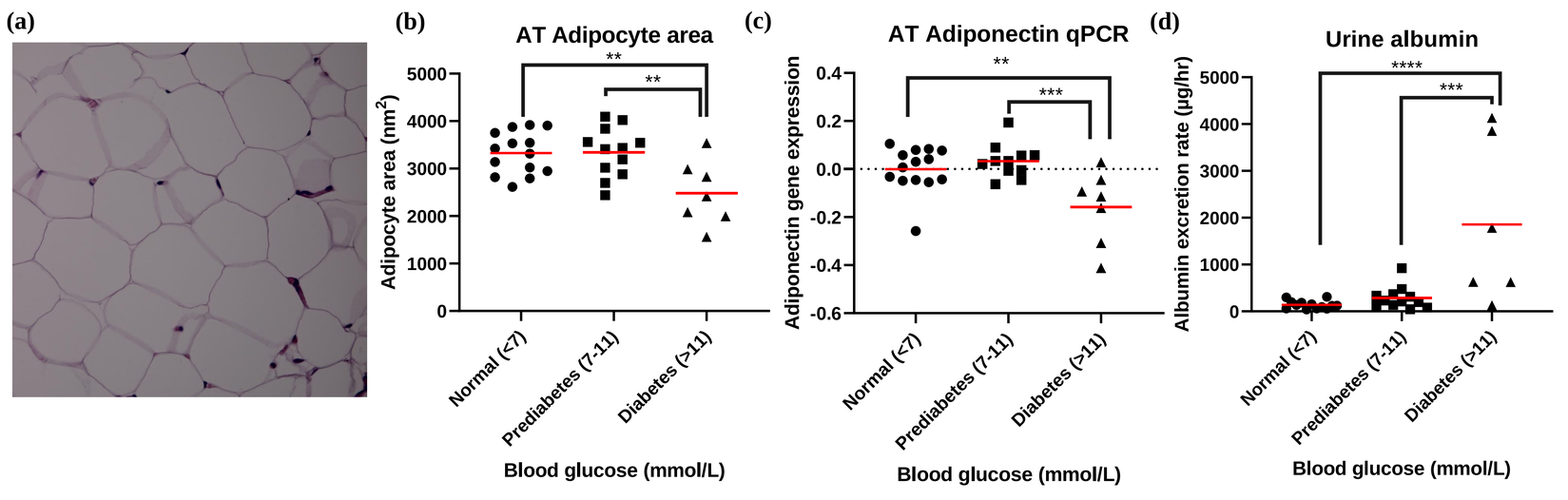

Figure 1: (a) Histological AT image (40x magnification) **(b)** Adipocyte area **(c)** AT adiponectin gene expression **(d)** Urine albumin excretion rate (µg/hr). Red lines indicate mean. ** denotes $p < 0.01$, ** denotes $p < 0.001$, **** denotes $p < 0.0001$

Figure 2: Ultrastructural features of glomerular filtration barrier

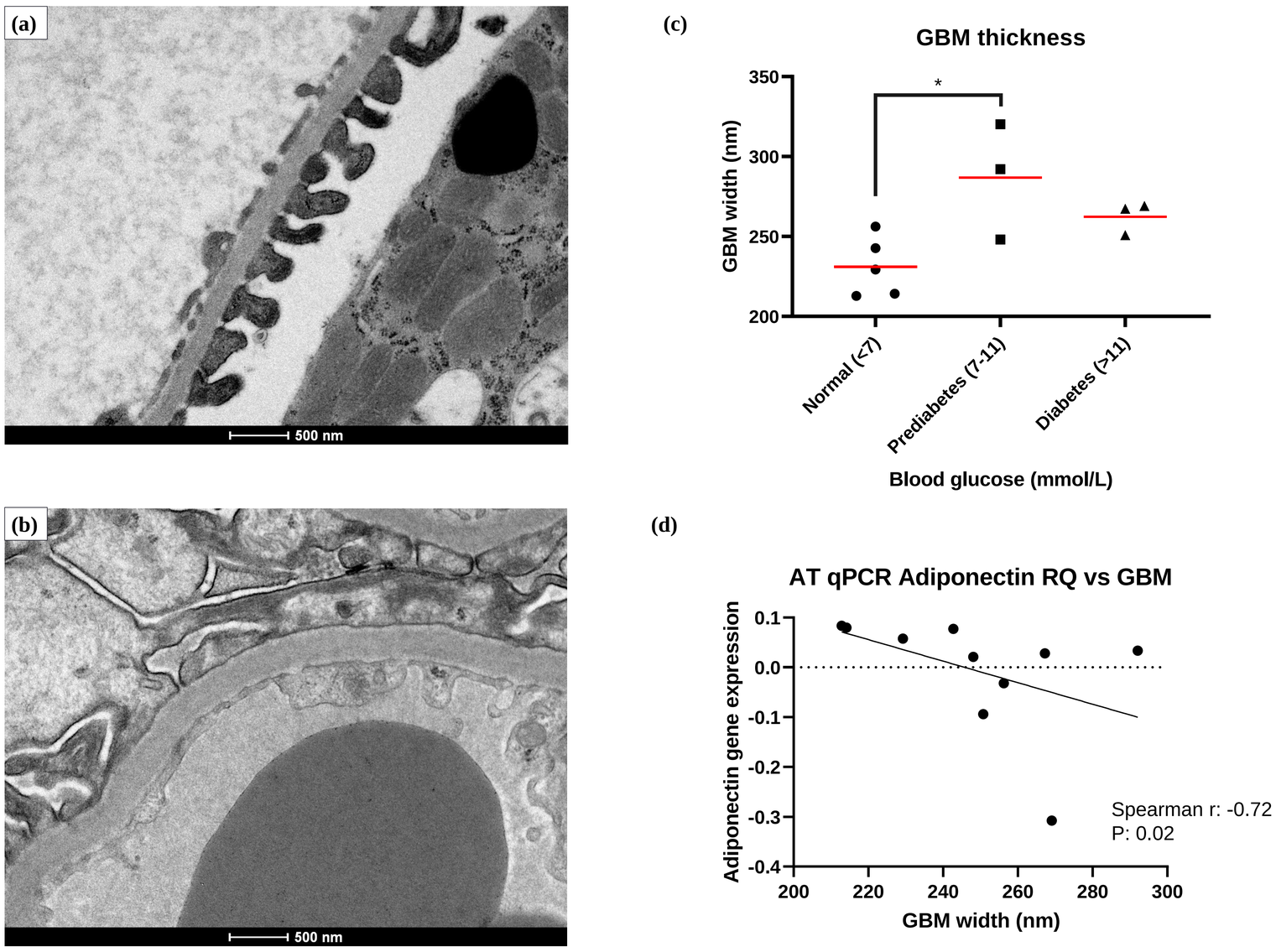

Figure 2: (a) & (b) Glomerular capillary loops from animal 2 and animal 10 respectively (20500x mag.). Note the podocyte effacement observable in Fig. 2 (b). **(c)** GBM thickness compared across glycaemia groups **(d)** Linear regression fit between GBM thickness and adiponectin gene expression. * denotes $p < 0.05$.

CONCLUSIONS

- The inclusion of normoglycaemic, prediabetic and diabetic animals permitted observation of clinical parameters in progressing T2DM.
- Prediabetic and diabetic animals presented clinical symptoms of DKD, including albuminuria and increased GBM thickness.
- The association between AT adiponectin expression and GBM thickness may provide evidence for a potential mechanistic role of a disrupted adipose-renal axis as a contributor to DKD progression.

References:

[1] Sharma, K., et al., 2008. Adiponectin regulates albuminuria and podocyte function in mice. *Journal of Clinical Investigation*, 118(5), pp.1645 – 1656.