Stereological quantification and whole-kidney 3D imaging for assessment of key pathological features in a uni-nephrectomised db/db mouse model of diabetic nephropathy

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INTRODUCTION AND AIM

Diabetic nephropathy (DN) is a long-term complication of diabetes characterized by increasing albuminuria and reduced kidney function. Approximately one third of patients with type 2 diabetes develop DN, with DN being the predominant cause of end-stage renal disease. To aid further understanding of disease mechanisms and development of new treatment options, translatable and stable rodent models that recapitulate features of human DN are essential.
Here we report stereological quantification and the use of light sheet microscopy to study whole-kidney glomerular changes in a mouse model of DN combining genetic diabetes (db/db mice) with uni-nephrectomy (UNx) to accelerate the development of DN.

STUDY DESIGN

In Fig. 1 female db/db or db/+ mice underwent sham surgery (Sham) or uni-nephrectomy (UNx) at the age of 7-8 weeks, while in Fig. 2 UNx surgery was performed in 18 weeks old male db/db mice. As detailed in Fig. 1, blood glucose and body weight was determined, and plasma and spot urine was collected throughout the study. Both studies were terminated when the mice were 24 weeks of age. At termination of female UNx db/db, Sham db/db and Sham db/+ mice, the right kidney was sampled for histological and stereological analyses, and next generation RNA sequencing, while the right kidney from male db/+ and UNx db/db mice was sampled for light sheet microscopy and 3D imaging. All data expressed as mean + SEM. Dunnett's test one-factor linear model applied on all data unless otherwise specified. Statistical analyses: *: p<0.05. **: p<0.01. ***: p<0.001.

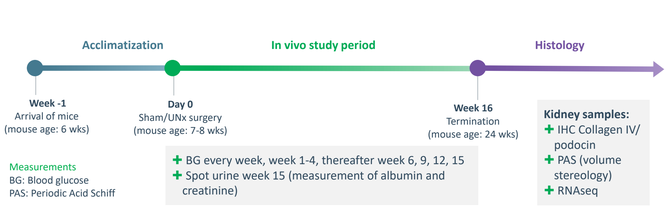


Figure 1 | Female Sham/db+ (n=8), Sham db/db (n=7-11) and UNx db/db (n=8-10) mice.

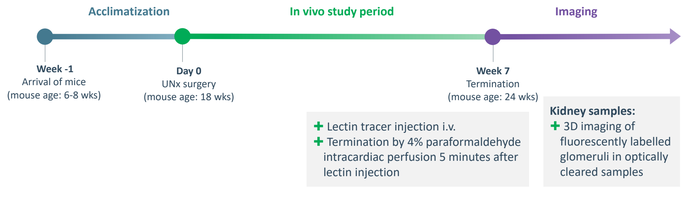


Figure 2 | Male db/+ (n=2) and UNx db/db (n=11) mice.

RESULTS

The db/db mice are diabetic and display increased ACR

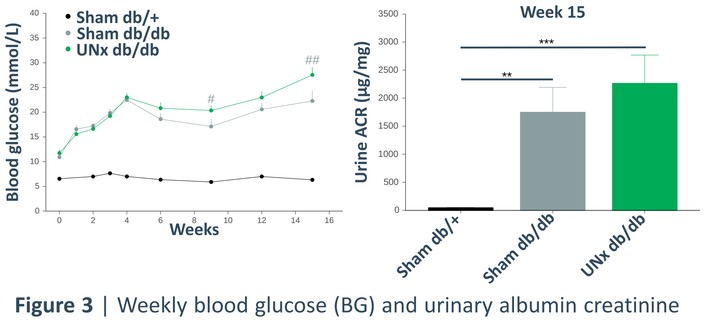


Figure 3 | Weekly blood glucose (BG) and urinary albumin creatinine ratio (ACR) measured after 15 weeks . Statistical analyses *left panel* | #: p<0.05, ##: p<0.01 vs UNX db/db. The UNx and Sham db/db mice had significantly higher BG at all time points compared to Sham db/+.

Kidney and glomerular hypertrophy in diabetic UNx mice

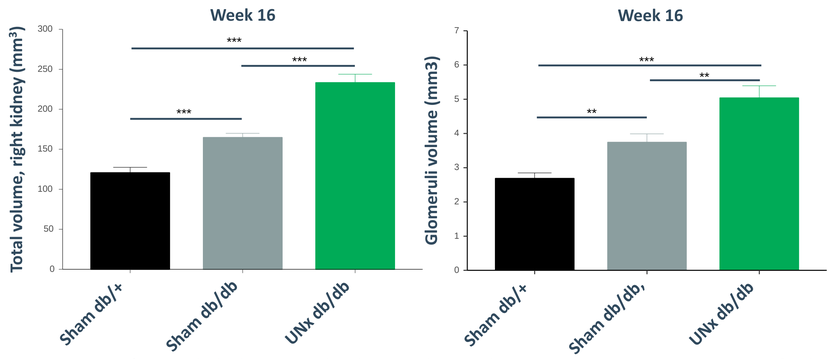


Figure 4 | Total kidney and glomerular volume at termination quantified stereologically (mm³) on PAS stained sections in the right kidney.

Increased glomerulosclerosis in diabetic UNx mice

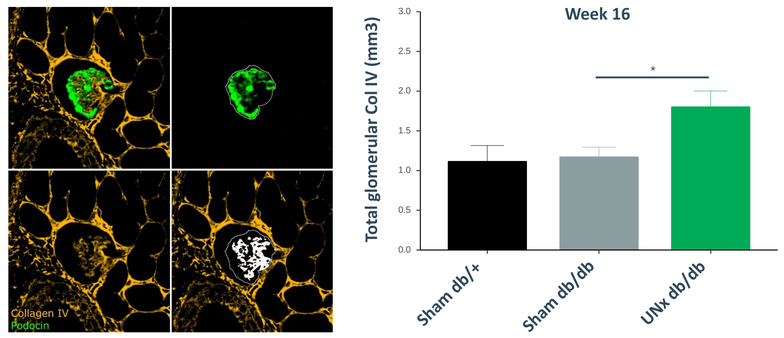


Figure 5 | Total glomerular Collagen IV was quantified stereologically (mm³) on podocin/collagen IV double stained sections in the kidney after 16 weeks. Podocin (green, podocytes) and Collagen IV (yellow, fibrosis) double staining was used to identify glomerulosclerosis (white overlay).

Regulated kidney injury and podocyte- and tubular-specific genes

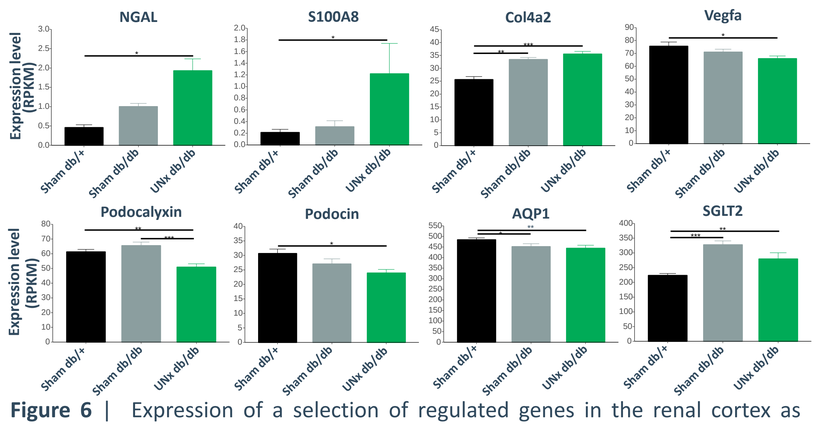


Figure 6 | Expression of a selection of regulated genes in the renal cortex as assessed by next generation RNA sequencing. P-values are adjusted for multiple comparisons.

3D imaging of kidneys from lectin_594 dosed db/+ and UNx db/db mice

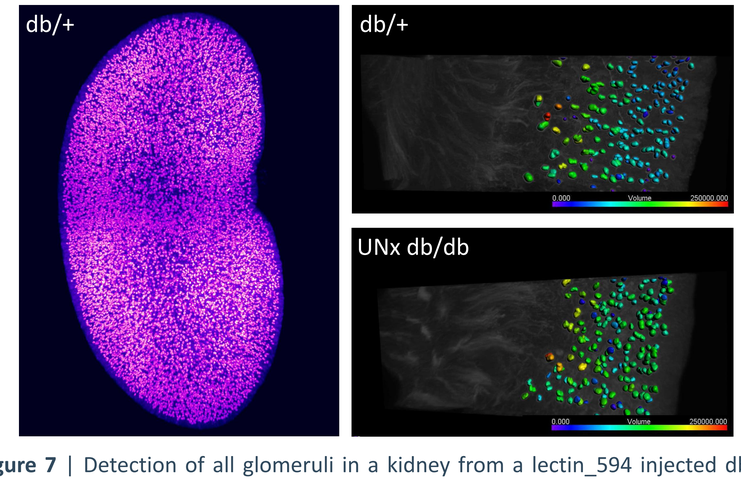
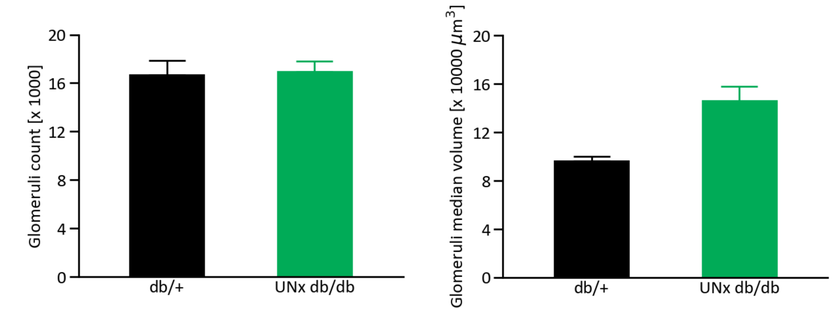


Figure 7 | Detection of all glomeruli in a kidney from a lectin_594 injected db/+ mouse scanned using light sheet microscopy. Representative color coded volume rendering of individual glomeruli shows a shift in the size distribution of glomeruli in the outer cortex of UNx db/db kidneys as compared to db/+ controls.

Increased median glomerulus size in diabetic UNx mice



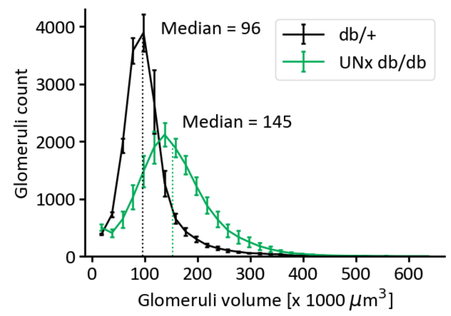
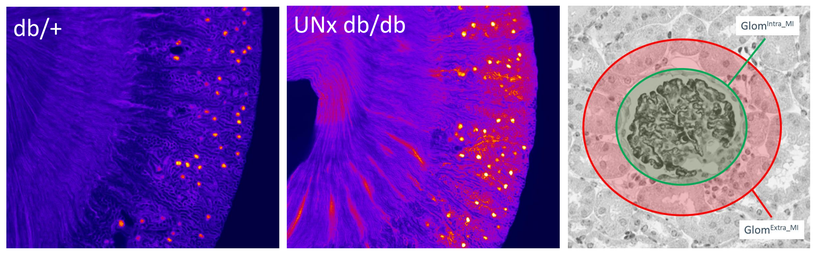


Figure 8 | The total number of glomeruli and size of all glomeruli in each kidney was calculated using an algorithm modified from Klingberg et al., JASN 2016. The total number of glomeruli per kidney was approximately 16.000 in both groups. However, the median size of glomeruli increased from 96 (x1000 mm³) in db/+ mice to 145 (x1000 mm³) in UNx db/db.

Glomerular filtration is impaired in diabetic UNx mice



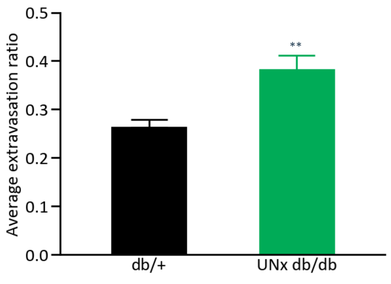


Figure 9 | Intravenous injection with the Lectin_594 dye in the kidneys of UNx db/db mice resulted in an unexpected accumulation of the dye in the cortex. To test if this observation can be used to measure impaired glomerular filtration in situ, we developed an algorithm to detect the Lectin_594 signal in the glomeruli and in the surroundings of the glomeruli (MI: mean intensity). The extravasation of lectin_594 in the UNx db/db mice was significantly increased compared to db/+ controls.

Video: 3D imaging showing all glomeruli in a whole kidney from a diabetic mice dosed with lectin. https://www.youtube.com/watch?v=pjS8YcP3pO0

CONCLUSION

The UNx db/db model of DN is characterized by:

- ✓ **Progressive albuminuria**
- ✓ **Kidney hypertrophy**
- ✓ **Increased glomerular volume, but not number**
- ✓ **Increased glomerulosclerosis**
- ✓ **Regulation of gene expression in the renal cortex incl. glomerular and tubular markers.**

We have successfully applied stereological quantification and light sheet microscopy with 3D imaging to assess renal and glomerular hypertrophy in uni-nephrectomised db/db mice.

These methods offer a new approach for high-resolution evaluation of key glomerular markers of DN