3D kidney imaging for assessment of glomerular number and size in a mouse model of diabetic nephropathy

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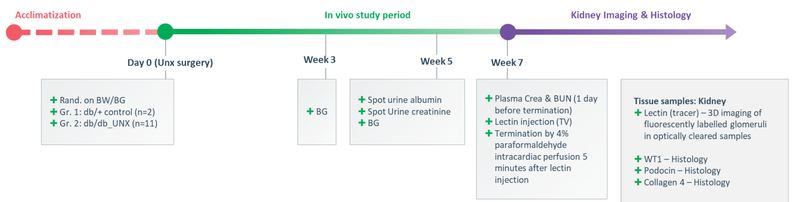
BACKGROUND AND AIMS

Diabetic nephropathy (DN) is a major long-term complication of diabetes characterized by hypertrophy of the kidney and reduced kidney function. To facilitate a rapid and unbiased evaluation of drug efficacy on glomerular size and number in preclinical studies, we investigated the use of light sheet microscopy as a new high-end 3D methodology to study glomerular changes in whole kidneys. Diabetic db/db mice subjected to unilateral nephrectomy (UNX) was used as a model.

STUDY DESIGN

Unilateral nephrectomy (UNX) was performed in diabetic db/db mice to accelerate the development of nephropathy. The surgery was performed in 18 weeks old male db/db mice, which were terminated 6 weeks later. To determine the effect of accelerated DN on glomerular morphology, mice were injected with Lectin_594 prior to termination and the intact kidneys were scanned using light sheet microscopy. Using 3D image analysis, the total number of glomeruli was quantified, and glomeruli segmented according to their individual size using an algorithm modified from Klingberg et al., 2017. Finally, we tested if kidneys subjected to light sheet microscopy were compatible with traditional immunohistochemistry.



RESULTS

The db/db_UNX mice are diabetic and display impaired kidney function

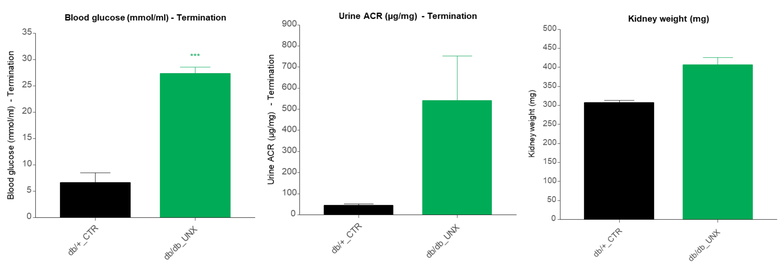


Figure 1 | At termination the blood glucose levels varied significantly between the non-diabetic db/+ control mice and db/db_UNX mice. The db/db_UNX mice also had increased urinary albumin-to-creatinine ratio (ACR) and kidney weight. Data are + SEM. Dunnett's test one-factor linear model ***: P < 0.001 compared to db/+_CTR

3D Imaging of kidneys from lectin_594 dosed mice

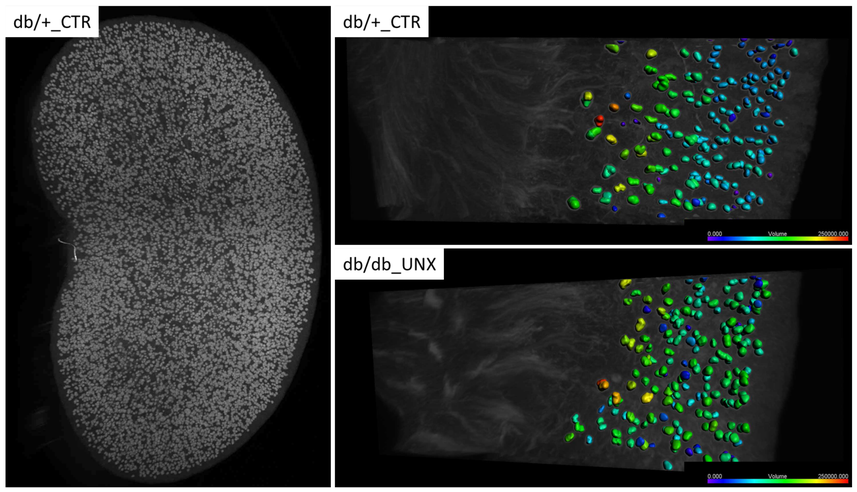


Figure 2 | Detection of all glomeruli in a kidney from a lectin_594 injected db/+ mouse scanned using Light sheet microscopy. Representative color coded volume rendering of individual glomeruli shows a shift in size (blue-green-red) of glomeruli in db/db_UNX kidneys as compared to the db/+ controls

Increased glomeruli median size in db/db_UNX mice

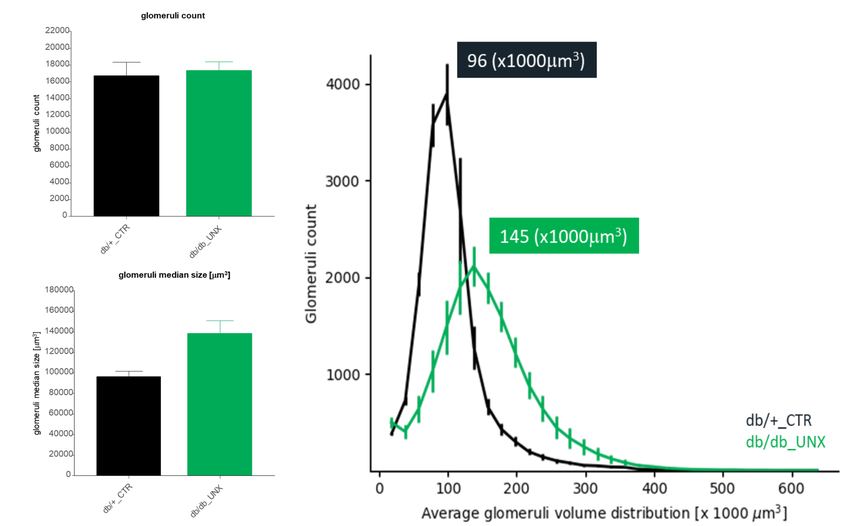


Figure 3 | Using the algorithm modified from Klingberg et al., 2017 we calculated the number and size of all glomeruli in each kidney. The total number of glomeruli per kidney was approximately ~16000 in both groups which corresponds well with previous reports. However, the median size of glomeruli increased from 96 (x1000µm³) in db/+_CTR mice to 145 in db/db_UNX.

Glomeruli filtration is impaired in db/db_UNX mice

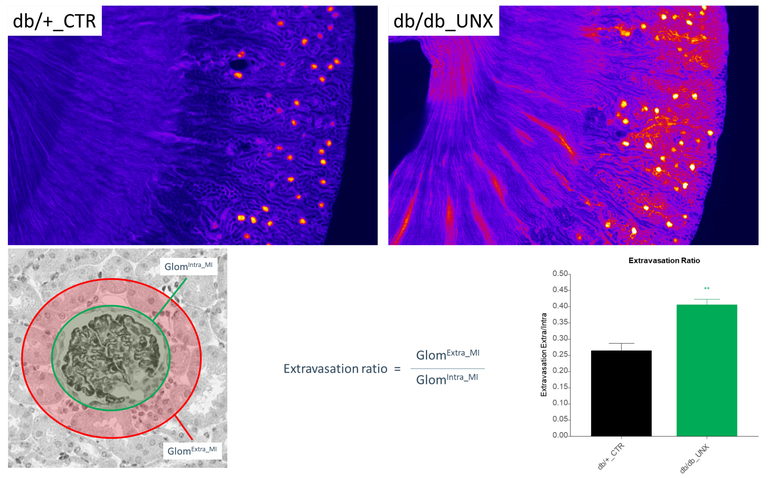


Figure 4 | Following IV injection the Lectin_594 dye was allowed to distribute in the body for approximately 5 minutes. In the kidneys from db/db_UNX mice this resulted in an unexpected accumulation of the dye in the cortex. To test if this observation can be used to measure impaired glomeruli filtration *in situ*, we developed an algorithm to detect the 594 signal surrounding the glomeruli. We found that the extravasation of lectin_594 the in the db/db_UNX mice is significantly increased compared to db/+ controls. Data are + SEM. Dunnett's test one-factor linear model **: P < 0.01 compared to bb/+_CTR (One outlier was removed from the db/db_UNX group)

Kidneys processed for light sheet microscopy are still amenable to traditional IHC

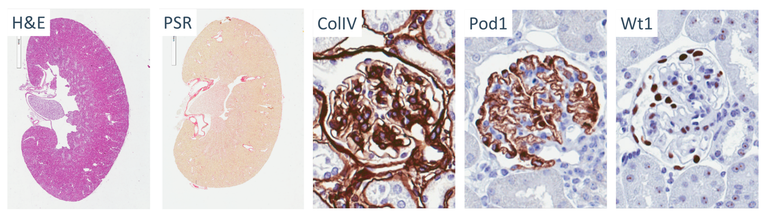


Figure 5 | To test if the reagents used for light sheet microscopy interfere with traditional immunohistochemical techniques, we processed kidneys for paraffin embedding and stained with H&E and PSR. We also tested a number of antibodies recognizing ColIV, Pod1 and WT1, markers associated with glomeruli function and biology.

CONCLUSION

- We have successfully applied light sheet microscopy to assess renal and glomerular hypertrophy at the whole organ level in uni-nephrectomised db/db mice.
- The method offers a new approach for evaluating changes in key glomerular markers of DN
- Glomeruli size, but not number, are affected in the db/db UNx model of DN

- *To see a 3D movie of a lectin labelled kidney see our homepage (www.gubra.dk)*