Unbiased Stereological Quantification of Key Features of Diabetic Nephropathy

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INTRODUCTION AND AIM

Diabetic nephropathy (DN) is a serious long-term complication of diabetes. There is no curative treatment of DN, and the lack of knowledge about the mechanisms leading to DN hampers the development of efficient therapies.

In order to develop a more precise assessment of translatable nephrotic changes, we used novel and refined image analyses and stereological methods to estimate key histopathological features of DN in a DN mouse model combining genetic diabetes (db/db mice) with uninephrectomy (UNx). The data are considered of high value for future preclinical drug development efforts.

Study design and groups

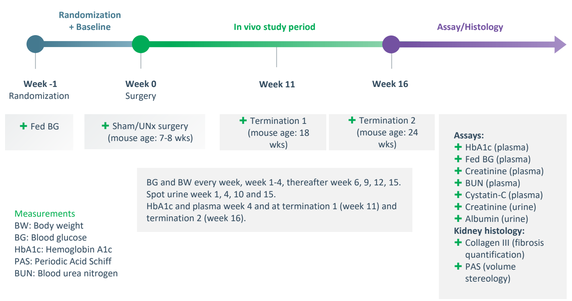


Figure 1 | Female db/db or db/+ mice underwent sham surgery (sham) or uninephrectomy (Unx) at the age of 7-8 weeks. Through 11-16 weeks, blood glucose and body weight was determined and plasma and spot urine was collected as detailed in the figure. At termination, plasma was sampled to allow detailed biochemical determinations. Also, kidneys were isolated for histology and stereology. All data are expressed as mean + SEM. n=6-29 per group. Dunnett's test one-factor linear model applied on all data. Statistical analyses in Figures 2b and 3-7: *: p<0.05. **: p<0.01. ***: p<0.001.

Color	Group characteristic	Weeks after surgery	Surgery	Group name
●	Non-diabetic control	11 or 16	Sham	Sham db/+
●	Diabetic control	11 or 16	Sham	Sham db/db
●	Diabetic UNx	11 or 16	UNx	UNx db/db

RESULTS

UNx does not affect body weight or blood glucose in diabetic mice

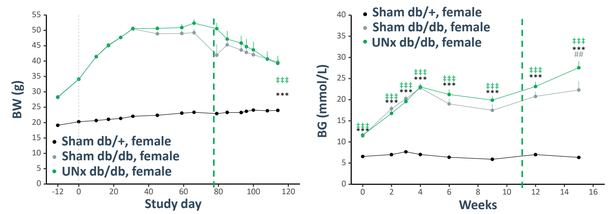


Figure 2a | Weekly body weight (BW, g) and blood glucose (BG, mmol/L). Green dashed lines indicate termination of the first group of mice (11 weeks). ***: p<0.001 vs sham db/db. ##: p<0.01 vs UNX db/db. ‡‡‡: p<0.001 vs sham db/+.

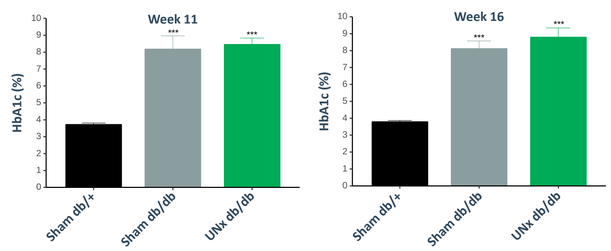


Figure 2b | Hemoglobin A1c (HbA1C) at termination week 11 and week 16.

Uninephrectomy increases kidney hypertrophy in diabetic mice

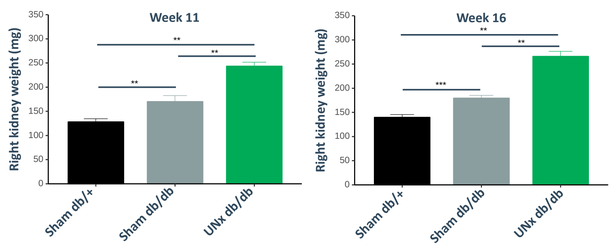


Figure 3 | Kidney weight (mg) at termination week 11 and week 16.

Albuminuria in db/db mice

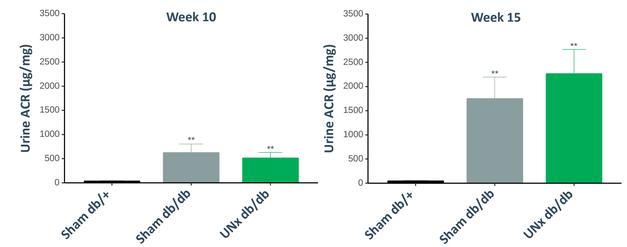


Figure 4 | Urinary albumin creatinine ratio (ACR) measured after 10 and 15 weeks.

Increased glomerular volume in diabetic UNx mice

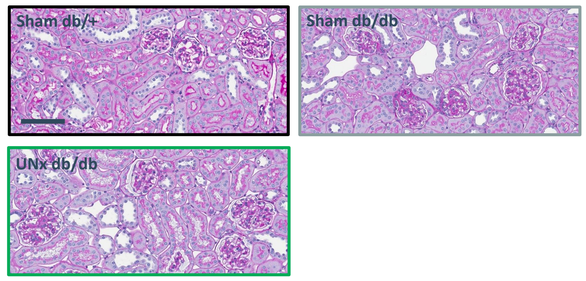


Figure 5a | Representative PAS stained images from sham db/+, sham db/db, and UNx db/db mice. Scale bar = 100µm.

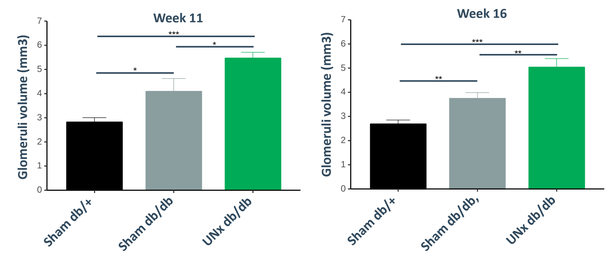


Figure 5b | Total glomerular volume was quantified stereologically (mm^3) on PAS stained sections in the right kidney after 11 and 16 weeks.

UNx increases total Collagen III in the kidney

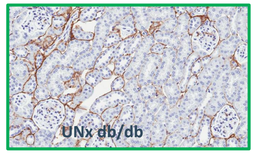
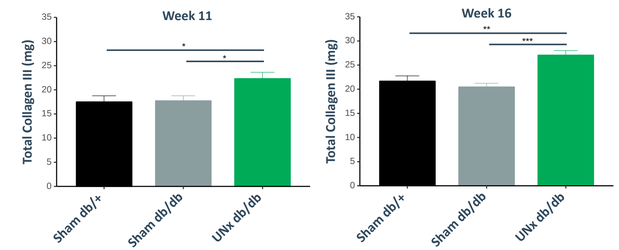


Figure 6 | left | Collagen III staining in kidney section from UNx db/db mouse. | Bottom | Total collagen III in the right kidney at week 11 (left) and week 16 (right).



Increased glomerulosclerosis in diabetic UNx mice

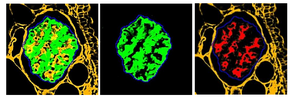


Figure 7 | Podocin (green, podocytes), Collagen IV (yellow, fibrosis) double stainings were used to identify glomerulosclerosis (red overlay).

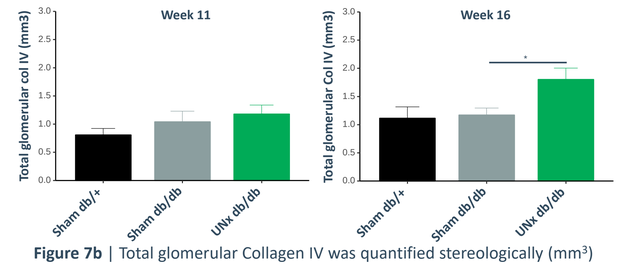


Figure 7b | Total glomerular Collagen IV was quantified stereologically (mm^3) on podocin/collagen IV double stained sections in the right kidney after 11 and 16 weeks.

CONCLUSION

- ✓ UNx does not affect body weight or blood glucose in female db/db mice
- ✓ The UNx db/db mouse model displays key features of diabetic nephropathy, incl. progressive albuminuria, kidney hypertrophy, kidney fibrosis, increased glomerular volume, and increased glomerulosclerosis
- ✓ Stereological quantification refines the use of the db/db UNx model in diabetic nephropathy research