Renal cell-type associated therapeutic effects of semaglutide in a mouse model of hypertension accelerated diabetic kidney disease

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Background & Aim

Obesity, hyperglycemia and hypertension are critical risk factors for development of diabetic kidney disease (DKD). While emerging evidence suggests that glucagon-like peptide-1 receptor (GLP-1R) agonists improve cardiovascular and renal outcomes in type 2 diabetes patients, their mode of action is presently unclear. Using paired bulk and single-nucleus RNA sequencing (RNAseq), we profiled renal transcriptome signatures of the long-acting GLP-1R agonist semaglutide alone and in combination with the ACE inhibitor lisinopril in a model of hypertension-accelerated, advanced DKD facilitated by adeno-associated virus-mediated renin overexpression (ReninAAV) in uninephrectomized (UNx) female db/db mice.

Methods

Seven weeks after ReninAAV administration and six weeks post-UNx, ReninAAV UNx *db/db* mice were administered (q.d.) vehicle, semaglutide (30 nmol/kg, s.c.) or semaglutide (30 nmol/kg, s.c.) + lisinopril (30 mg/kg, p.o.) for 11 weeks. Endpoints included blood pressure, urine biochemistry, kidney histopathology as well as paired bulk and single-nucleus RNA seq.
Cell type deconvolution was performed by referencing expression of treatment-affected genes across all major kidney cell types using single nuclei RNAseq.

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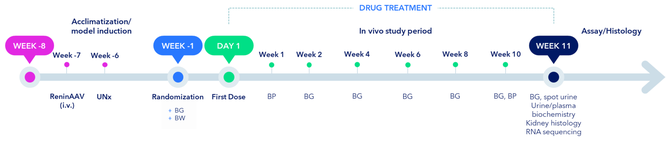
1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	ReninAAV Unx *db/db*	Female	15	**Vehicle**	SC	QD	5 ml/kg	-
2	ReninAAV Unx *db/db*	Female	15	**Semaglutide**	SC	QD	5 ml/kg	**30 nmol/kg**
3	ReninAAV Unx *db/db*	Female	14	**Semaglutide + Lisinopril**	SC + PO	QD	5 ml/kg	**30 nmol/kg + 30 mg/kg**

2 Improved metabolic parameters

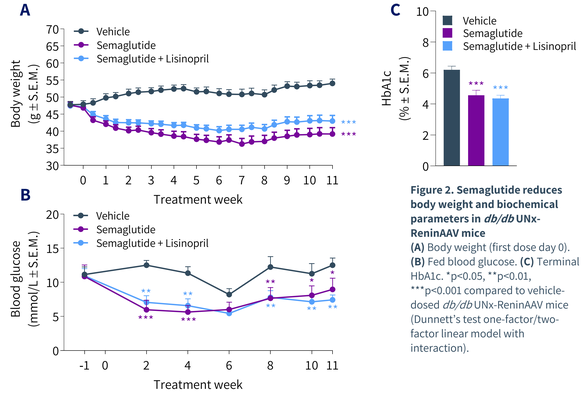


Figure 2. Semaglutide reduces body weight and biochemical parameters in *db/db* UNx-ReninAAV mice
(A) Body weight (first dose day 0). **(B)** Fed blood glucose. **(C)** Terminal HbA1c. *p<0.05, **p<0.01, ***p<0.001 compared to vehicle-dosed *db/db* UNx-ReninAAV mice (Dunnett's test one-factor/two-factor linear model with interaction).

3 Improvements in hypertension and albuminuria

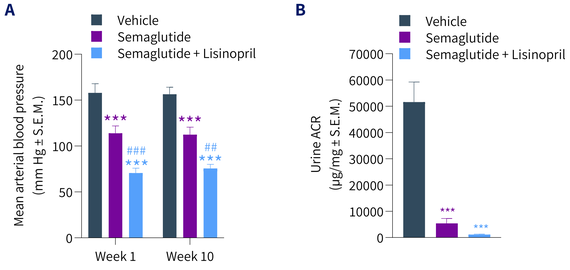


Figure 3: Semaglutide markedly improves hypertension and albuminuria in *db/db* UNx-ReninAAV mice
(A) Mean arterial blood pressure measured in treatment week 1 and 10. **(B)** Albumin-to-creatinine ratio (terminal spot urine samples). ***p<0.001 vs vehicle-dosed *db/db* UNx-ReninAAV mice. ##p<0.01, ###p<0.001 vs. semaglutide (Dunnett's test two-factor linear model with interaction).

4 Reduced glomerulosclerosis

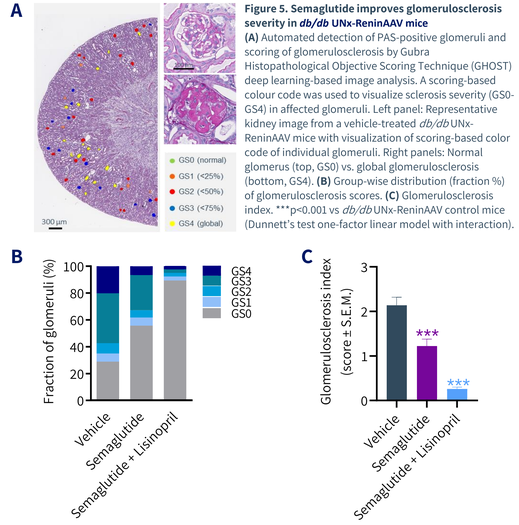


Figure 5. Semaglutide improves glomerulosclerosis severity in *db/db* UNx-ReninAAV mice
(A) Automated detection of PAS-positive glomeruli and scoring of glomerulosclerosis by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. A scoring-based colour code was used to visualize sclerosis severity (GS0-GS4) in affected glomeruli. Left panel: Representative kidney image from a vehicle-treated *db/db* UNx-ReninAAV mice with visualization of scoring-based color code of individual glomeruli. Right panels: Normal glomerus (top, GS0) vs. global glomerulosclerosis (bottom, GS4). **(B)** Group-wise distribution (fraction %) of glomerulosclerosis scores. **(C)** Glomerulosclerosis index. ***p<0.001 vs *db/db* UNx-ReninAAV control mice (Dunnett's test one-factor linear model with interaction).

5 Renal cell-type associated transcriptome changes

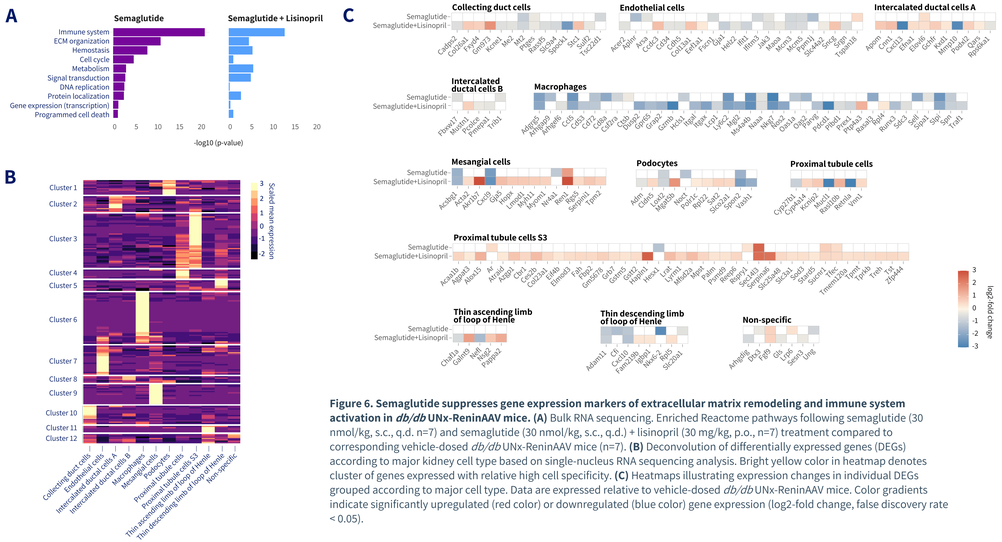


Figure 6. Semaglutide suppresses gene expression markers of extracellular matrix remodeling and immune system activation in *db/db* UNx-ReninAAV mice. (A) Bulk RNA sequencing. Enriched Reactome pathways following semaglutide (30 nmol/kg, s.c., q.d. n=7) and semaglutide (30 nmol/kg, s.c., q.d.) + lisinopril (30 mg/kg, p.o., n=7) treatment compared to corresponding vehicle-dosed *db/db* UNx-ReninAAV mice (n=7). **(B)** Deconvolution of differentially expressed genes (DEGs) according to major kidney cell type based on single-nucleus RNA sequencing analysis. Bright yellow color in heatmap denotes cluster of genes expressed with relative high cell specificity. **(C)** Heatmaps illustrating expression changes in individual DEGs grouped according to major cell type. Data are expressed relative to vehicle-dosed *db/db* UNx-ReninAAV mice. Color gradients indicate significantly upregulated (red color) or downregulated (blue color) gene expression (log2-fold change, false discovery rate < 0.05).

Conclusion

Semaglutide alone and in combination with lisinopril:

+ Reduces body weight, blood glucose and HbA1c
+ Markedly improves hypertension and albuminuria
+ Markedly reduces glomerulosclerosis
+ Improves renal transcriptome signatures

These findings support nephroprotective effects of semaglutide in DKD, highlighting the applicability of the db/db UNx-ReninAAV mouse model in preclinical drug development.

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