Hyperinsulinemic milieu elicits glomerular podocyte impairment and dysfunction via inducing GSK3β hyperactivity

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**Background:** Epidemiological evidence suggests that hyperinsulinemia or insulin resistance is a significant risk factor for the development of diabetic complications such as DKD. However, whether hyperinsulinemia per se plays a causative role in the development of diabetic kidney injury is unknown and was explored here.

**Methods:** Pre-diabetic db/db mice were examined for serum insulin levels, urinary albumin to creatinine ratios and renal histology. Conditionally immortalized mouse podocytes were cultured under non-permissive conditions and exposed to high ambient insulin conditions, following GSK3β silencing, ectopic expression of a constitutively active GSK3β mutant (S9A), or treatment with a small molecule GSK3β inhibitor tideglusib. Podocyte injury was assessed and signaling pathways examined.

**Results:** In pre-diabetic db/db mice, hyperinsulinemia was evident and associated with microalbuminuria and early signs of podocyte impairment, including diminished expression of homeostatic marker proteins like synaptopodin, as compared with db/m littermates. In vitro, prolonged exposure of differentiated podocytes to high ambient insulin induced podocytopathic changes, including cellular hypertrophy, loss of synaptopodin, and disruption of actin cytoskeleton integrity. This was associated with a desensitized insulin signaling and diminished inhibitory phosphorylation of GSK3β, denoting GSK3β hyperactivity. In pre-diabetic db/db mice, GSK3β hyperactivity was confirmed in glomerular podocytes, correlating with the level of hyperinsulinemia or microalbuminuria. In cultured podocytes, ectopic expression of S9A caused podocyte hypertrophy and podocytopathic changes, reminiscent of the harmful effect of the hyperinsulinemic milieu. Conversely, GSK3β knockdown mitigates podocyte injury elicited by hyperinsulinemic milieu. This protective effect was mimicked by the small molecule inhibitor tideglusib.

**Conclusion:** GSK3β hyperactivity is required and sufficient for Hyperinsulinemic milieu-elicited glomerular podocyte impairment and dysfunction.