PREVENTION OF SYSTEMIC HYPERTENSION AND KIDNEY INJURY IN DIABETIC MICE BY INSULIN INHIBITION OF ANGIOTENSINOGEN GENE EXPRESSION VIA DOWN-REGULATION OF NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2

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Objectives:

Angiotensinogen (Agt) is the sole precursor of all angiotensins. We have previously reported that high glucose stimulates renal Agt gene expression via nuclear factor erythroid 2-related factor 2 (Nrf2) in type 1 diabetic Akita mice (Diabetes 2014). The present studies investigated whether insulin inhibits renal angiotensinogen (Agt) gene expression via down-regulation of Nrf2 and thus prevents systemic hypertension in type 1 diabetic Akita mice.

Methods:

Male Akita mice were studied from 10-16 weeks of age with or without insulin implants (Linβit) from week 12. Age- and sex-matched non-Akita littermates served as wild type (WT) controls. We also did insulin clamp studies in WT mice (age: 12-14 weeks) using constant intravenous insulin infusion (10 mU/min/kg) plus variable glucose infusion rate to clamp glycemia at ~ 8 mM for 3 hours (euglycemic-hyperinsulinemic clamp). WT mice infused with saline served as controls. Kidneys were analyzed for Nrf2, Agt, and heterogeneous nuclear ribonucleoprotein F and K (hnRNP F/K) expression by immunohistochemistry. mRNA and protein expression in renal proximal tubules (RPTs) were evaluated by real time-qPCR and Western blotting, respectively. Immortalized rat renal proximal tubular cells (IRPTCs), stably transfected with plasmid pGL4.20 and containing either rat Nrf2, Agt, hnRNP F or hnRNP K gene promoter, were also studied *in vitro.*

Results:

In Akita mice, insulin treatment normalized blood glucose levels, reversed systemic hypertension and renal oxidative stress, inhibited renal Nrf2 and Agt gene expression, whereas it enhanced hnRNP F/K gene expression and attenuated renal hypertrophy, glomerular hyperfiltration and tubulointerstitial fibrosis. In WT mice subjected to euglycemic-hyperinsulinemic clamp, renal Agt and Nrf2 gene expression was down-regulated, whereas hnRNP F/K gene expression was up-regulated as compared to saline-infused control WT mice. *In vitro*, insulin inhibited Nrf2 and Agt gene promoter activity, and stimulated hnRNP F/K gene promoter activity in high glucose media via the p44/42 mitogen-activated protein kinase signaling pathway. Transfection with p44/42 MAPK small interfering RNA enhanced insulin inhibition of Agt and stimulated hnRNP F/K gene promoter activity in IRPTCs.

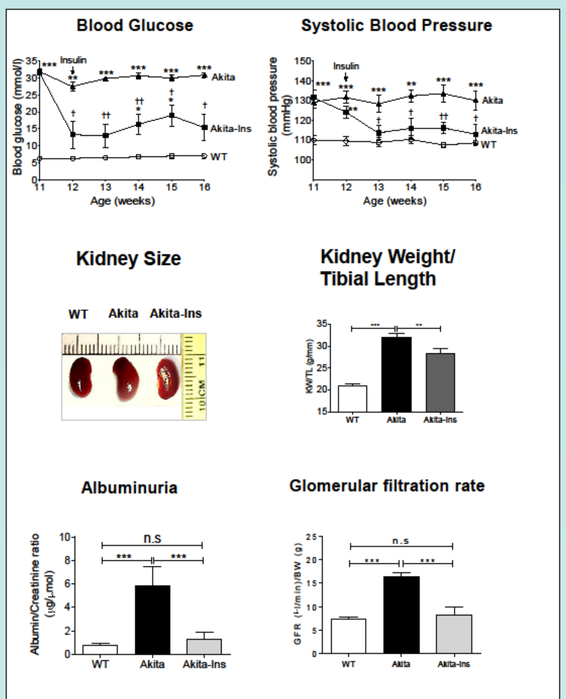

Figure 1

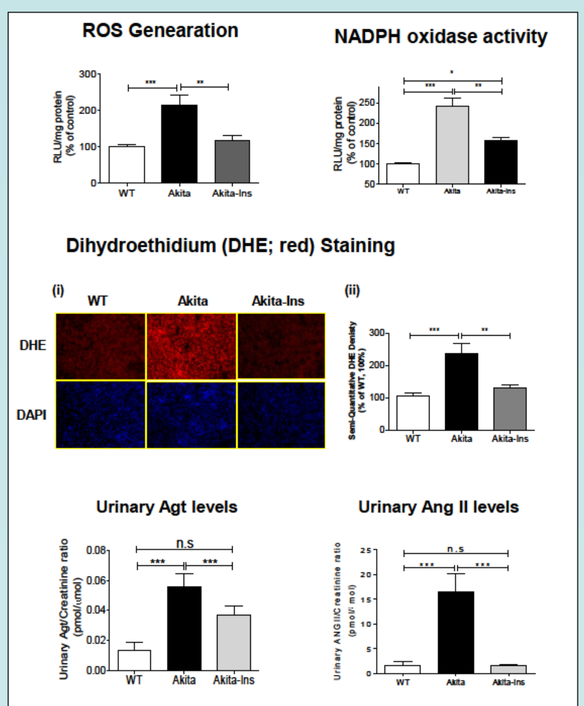

Figure 2

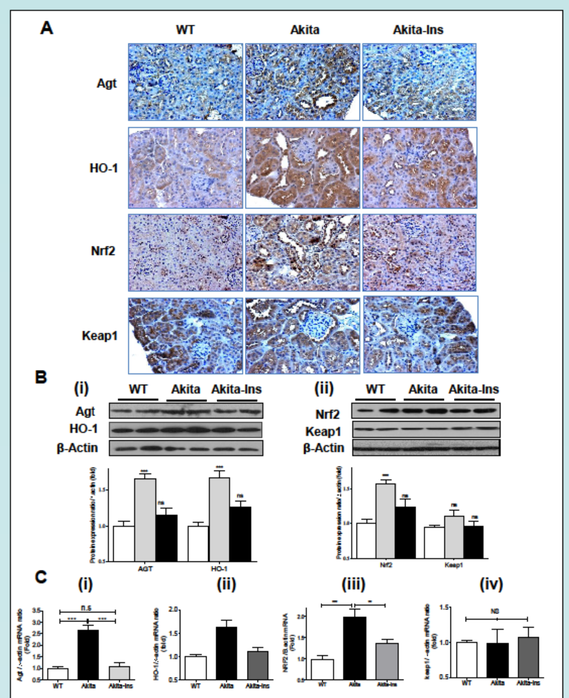

Figure 3

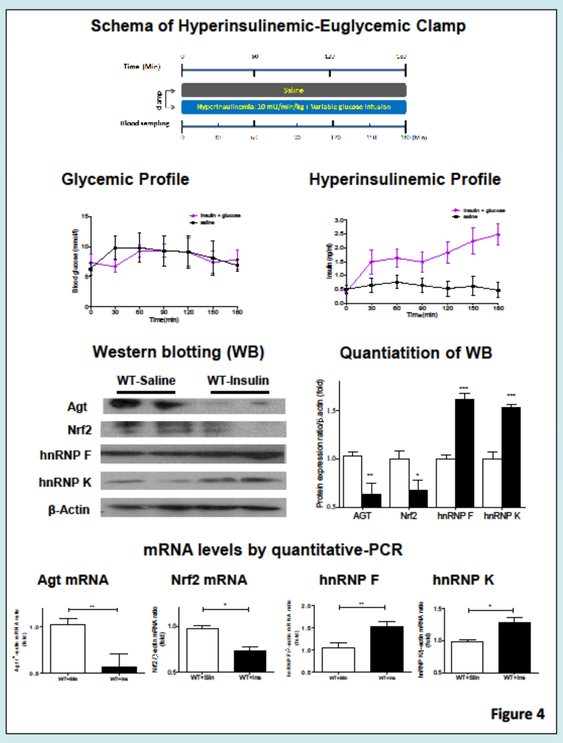

Figure 4

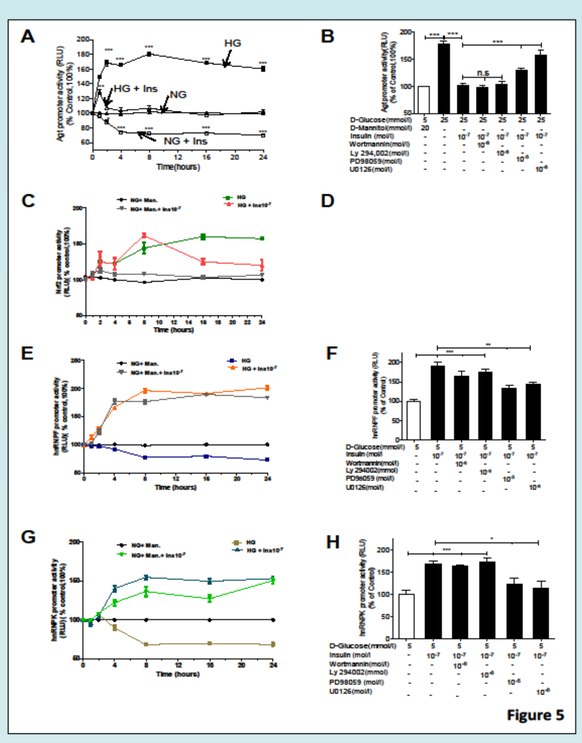

Figure 5

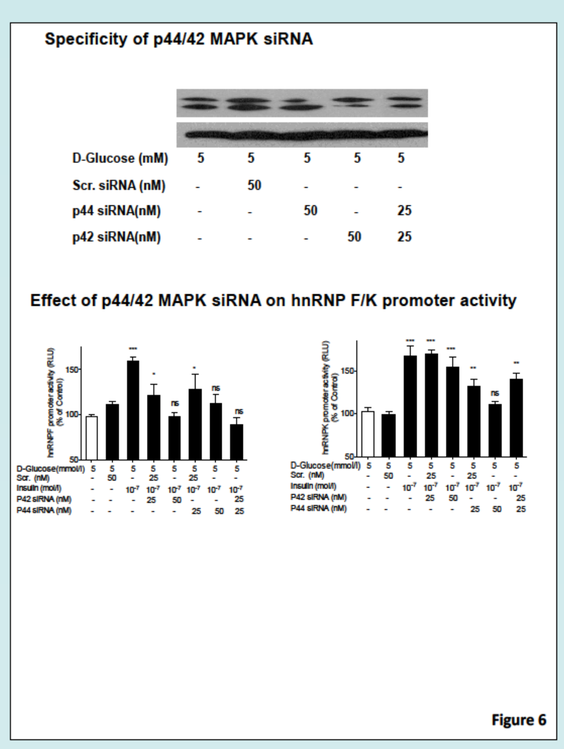

Figure 6

Conclusions:

Our data indicate that insulin prevents hypertension and attenuates kidney injury, at least in part, through suppressing renal Nrf2 and Agt gene transcription and up-regulating hnRNP F/K gene expression in diabetic Akita mice, independent of its glucose lowering effect.

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