Lower blood glucose and disrupted glucagon signaling in mice with liver-specific ablation of the Hepatocyte Nuclear Factor 4α (*Hnf4a*) gene.

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ABSTRACT

Aim: *Hnf4a* gene ablation in mouse liver causes hepatic steatosis, perturbs HDL structure and function and affects many pathways and genes related to glucose metabolism. Our aim was to investigate the role of liver HNF4A in glucose homeostasis.

Methods: Serum and tissue samples were obtained from *Alb-Cre;Hnf4a*$^{fl/fl}$ (H4LivKO) mice and their littermate *Hnf4a*$^{fl/fl}$ controls. Binding of HNF4A to DNA was assessed by chromatin immunoprecipitation (ChIP) assays. Fasting glucose and insulin serum levels, glucose tolerance, insulin tolerance and glucagon challenge tests were performed by standard procedures. Gene expression analysis was performed by quantitative real time PCR (qRT-PCR). Glucagon and insulin were detected by immunofluorescence on frozen pancreatic sections. Histological analysis for hematoxylin and eosin (H&E) and Masson trichrome were performed on paraffin embedded tissues by standard procedures.

Results: H4LivKO mice presented lower blood levels of fasting glucose, improved glucose tolerance, increased serum lactate levels and reduced response to glucagon challenge test compared to their control littermates. The levels of phospho-AKT were reduced in the liver of H4LivKO mice despite the increased serum insulin levels suggesting the development of insulin resistance. This is line with our gene expression analysis which revealed altered expression of genes involved in glycolysis and gluconeogenesis, including *Pck2*, *Pdk4*, *Pklr* and *Pcx*. Key genes involved in glycogen metabolism were downregulated in H4LivKO mice, including genes that are defective in glycogen storage diseases (*Gys2*, *Phka2*, *Pygl*). The expression of the gene encoding the glucagon receptor (*Gcgr*) was also markedly reduced in H4LivKO liver and ChIP assays showed specific and strong binding of HNF4A to the *Gcgr* promoter. Immunofluorescence for glucagon and insulin in pancreatic islets revealed alpha-cell hyperplasia indicating the disruption of the liver-α-cell axis. Glucose administration in the drinking water of H4LivKO mice beginning at four weeks of age resulted in an impressive extension of survival beyond 28 weeks compared to 6-8 weeks in mice that did not receive glucose. Masson trichrome staining of the liver showed a slight increase in the deposition of collagen at 6 weeks of age in H4LivKO mice but minor differences between control and H4LivKO after 24 weeks of glucose administration. Finally, transcriptomic analysis showed increase in the expression of genes associated with the progression of non-alcoholic fatty liver disease (NAFLD) in H4LivKO mice.

Conclusion: Our results reveal a novel role of liver HNF4A in controlling blood glucose levels via regulation of the glucagon receptor. In combination with the steatotic phenotype, our results suggest that H4LivKO mice could serve as a valuable model for studying glucose homeostasis in the context of NAFLD.