Fibroblast Growth Factor 15 (FGF15) Suppresses NASH Development in Mice

Campbell, MJ[1]; Kong, B[1]; Guo, GL[1]; Ernest Mario School of Pharmacy, [1]Rutgers University

Rutgers Ernest Mario School of Pharmacy

Rutgers Aresty Research Center for Undergraduates

ABSTRACT

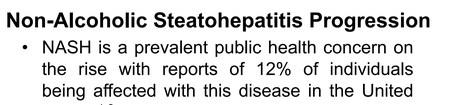
Non-Alcoholic Steatohepatitis (NASH) is a severe progression of Non-Alcoholic Fatty Liver Disease (NAFLD) in which liver steatosis, inflammation and hepatocyte damage occurs. Currently the role of Fibroblast Growth Factor 15 (FGF15) in NASH development is unclear.

This research determines the effect of intestinal FGF15 deficiency on NASH development using the conditional knockout (CKO) of intestinal *Fgf15* in a high fat diet (HFD)-induced NASH model in mice. Furthermore this research serves to investigate the potential of FGF15 as a novel drug target for NASH pharmacotherapies.

HYPOTHESIS

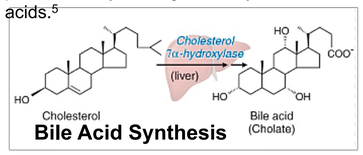
Intestinal *Fgf15* CKO mice fed a high fat diet (HFD) will experience an increase in FXR activity as well as an increase in bile acid synthesis, hepatic inflammation, and liver fibrosis

INTRODUCTION



Non-Alcoholic Steatohepatitis Progression

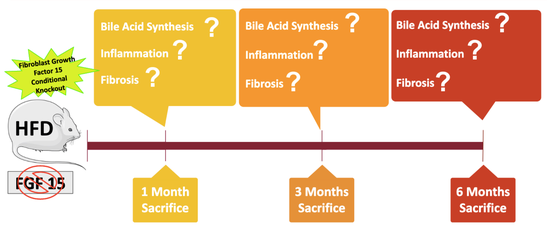
- NASH is a prevalent public health concern on the rise with reports of 12% of individuals being affected with this disease in the United States.[1,2]
- Patients with NASH are at a higher risk for end stage liver disease, liver failure, and hepatocellular carcinoma.[3]
- NASH-related cirrhosis has become the most common cause for liver transplants in the US, surpassing HCV. [4]
- **There is currently no FDA approved treatment, and life-style modification and bariatric surgery are the only choices.**
- NASH is caused by dysregulation of liver homeostasis.[1,5,6]
- Hepatocytes are major cells capable of eliminating cholesterol and do so predominantly through the synthesis of bile acids.[5]



- Bile acids are signaling molecules that regulate lipid and glucose metabolism.[5]
- Excess bile acids are toxic to cells as their detergent properties damage cell membranes leading to cell death and inflammation.[5]

- Previous research has found that Farnesoid X Receptor (FXR) protects the liver from NASH progression.[6]
- An intestinal FXR downstream target gene is fibroblast growth factor (FGF) 15/19. Currently, the role of FGF15 in NASH development is unclear.

METHODS



Treatment Paradigm: Male *Fgf15* CKO mice were fed either control chow (CC) or high fat diet (HFD) , and sacrificed at 1, 3, and 6 months for assessment of NASH progression.

Gene Expression: Total RNA was isolated from tissue samples using TRIzol reagent according to the manufacturer's instructions. The concentration of total RNA was determined by NanoDrop Spectrophotometry and integrity confirmed by gel electrophoresis. Gene expression was analyzed via quantitative real time PCR (qRT-PCR) using SYBR green chemistry. All data were normalized to β-Actin expression.

Statistics: One Way ANOVA and t-test were completed where appropriate using SigmaPlot 11 software. For gene expression: a value of $P<0.05$ was considered statistically significant and indicated by an asterisk (*). Bar graphs represent the mean±SD.

PROPOSED MECHANISM

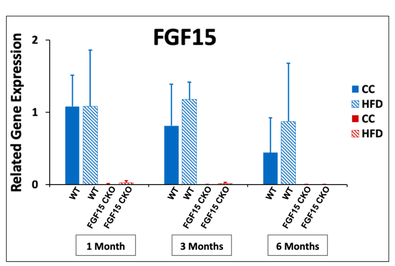


Figure 1. FGF15 mRNA levels were examined to confirm that the subjects were indeed intestinal *Fgf15* conditional knockouts.

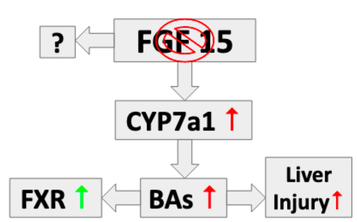


Figure 2. A proposed mechanism on the effects of intestinal *Fgf15* deficiency. A paradoxical increase in protective FXR activity balances with an increase in bile acid (BA) induced liver injury.

RESULTS: BA Synthesis

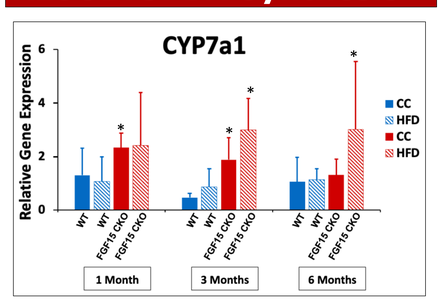


Figure 7. The mRNA levels of Cyp7a1 showed an increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 3 and 6 months

RESULTS: FXR Activity

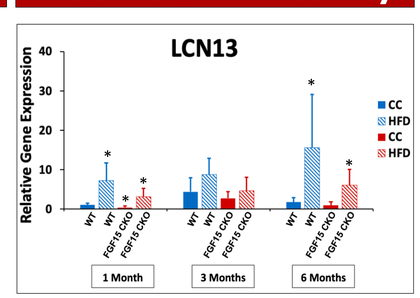


Figure 8. The mRNA levels of Lcn13 showed a significant decrease in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 1 and 6 months.

CONCLUSION

FGF15 Deficiency Assessment:

- Increased FXR activity, however, liver still exhibited NASH progression
- Increased bile acid synthesis
- Increased steatosis
- Increased inflammation and fibrosis

FGF15 serves as a vital player in gut-liver cross talk, most likely protecting the liver from NASH-related excess bile acid injury.

RESULTS: Hepatic FXR Activity

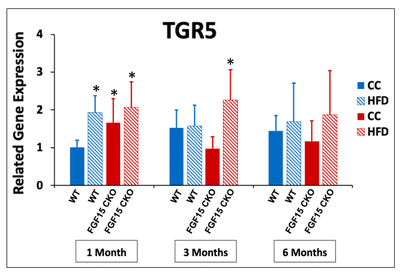


Figure 3. The mRNA levels of Tgr5 showed an increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 1 and 3 months

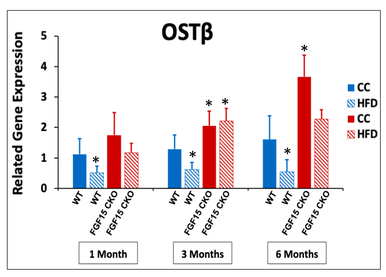


Figure 4. The mRNA levels of Ostb showed a significant increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 3 months

RESULTS: Inflammation

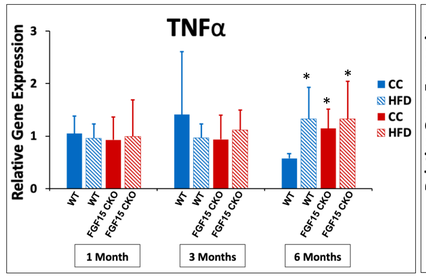


Figure 9. The mRNA levels of Tnfa showed a significant increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 6 months

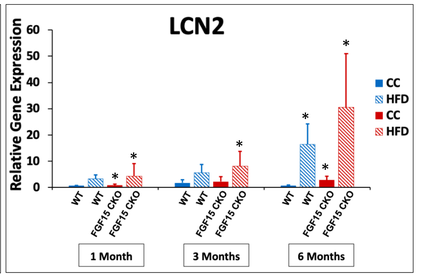


Figure 10. The mRNA levels of Lcn2 showed an increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 1, 3 & 6 months

RESULTS: Intestinal FXR Activity

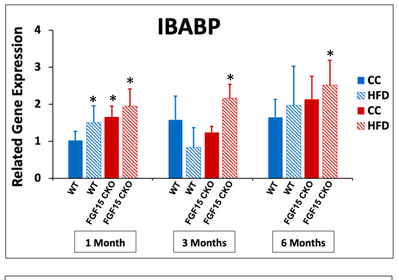


Figure 5. The mRNA levels of Ibabp showed an increase in expression levels in *Fgf15* CKO mice being fed a HFD at 1,3, & 6 months

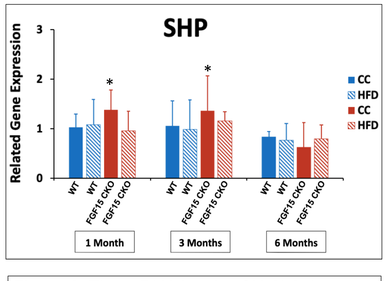


Figure 6. The mRNA levels of Nr0b2 that encodes Shp showed an increase in expression levels in *Fgf15* CKO mice compared to WT at 1 & 3 months

RESULTS: Fibrosis

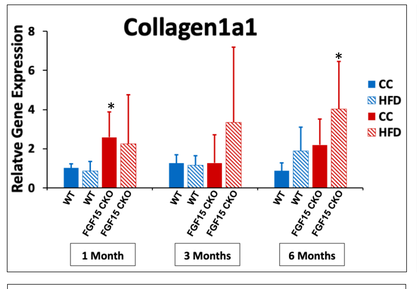


Figure 11. The mRNA levels of col1a1 showed an increase in expression levels in *Fgf15* CKO mice being fed a HFD at 1,3, & 6 months

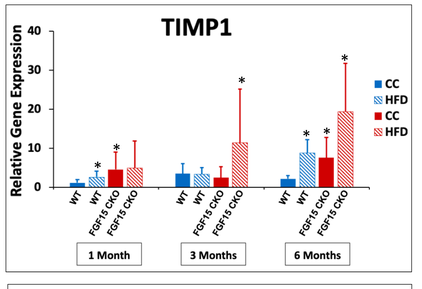
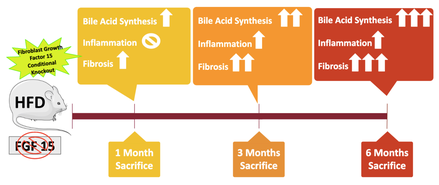


Figure 12. The mRNA levels of Timp1 showed an increase in expression levels in *Fgf15* CKO mice compared to WT being fed a HFD at 3 and 6 months



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Contact Info

For questions contact Michael J. Campbell
Email: mjc534@rutgers.edu