

# Berberine reduces inflammation, fibrosis, and dysregulation of bile acid and lipid metabolism in a mouse model of non-alcoholic fatty liver disease (NAFLD) at the transcriptional level



Andrew Nam<sup>1</sup>, Kaitlyn Jackson<sup>2</sup>, Dr. Huiping Zhou<sup>2</sup>

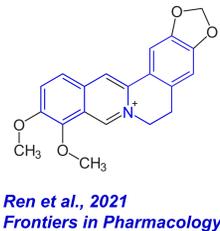
<sup>1</sup>VCU Medical Sciences Internship Program, Virginia Commonwealth University, Richmond, VA

<sup>2</sup>Department of Microbiology and Immunology, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA.

**Abstract:** Non-alcoholic fatty liver disease (NAFLD) is one of the fastest rising liver diseases. 10-25% of these patients develop non-alcoholic steatohepatitis (NASH), characterized by chronic inflammation and damage to the liver parenchymal tissue that increases the risk for liver cirrhosis and end-stage liver disease. Despite the rising need for novel therapies, no FDA-approved pharmacotherapies exist for NAFLD treatment. The current standard of care for NAFLD/NASH is physical exercise and caloric restriction. Berberine (BBR) is a traditional Chinese medicinal herb originally used to treat gastrointestinal upset. Our previous data shows that BBR interacts by regulating several proinflammatory and profibrotic signaling pathways through modulating ER stress in macrophages and hepatocytes. Based on these data, we hypothesized that Berberine will modulate proinflammatory and ER stress pathways to limit hepatic fibrosis, inflammation, and lipid accumulation in the high-fat diet mouse model of NAFLD. For this study, male and female mice (age 8-12 weeks) were fed either normal diet (control) or Western diet with sweet water (NASH) with or without BBR (50ug/g) for 21 weeks. A portion of liver tissue was processed for bulk RNA-sequencing (RNA-seq). After data conversion and normalization, the differentially expressed genes (DEGs) were calculated using the R package, EdgeR. Compared to NASH alone, NASH mice treated with BBR exhibited upregulation of many genes associated with bile acid and lipid metabolism. In addition, BBR downregulated WDSW-induced genes associated with hepatic fibrosis and inflammation, including neutrophil activation and ER stress to levels comparable to ND mice. This data indicates that BBR improves multiple parameters of NAFLD pathology by modulating key genes associated with steatohepatitis and fibrosis progression. While more research is necessary to determine the full scope of BBR's safety and efficacy, these encouraging results suggest BBR may be a viable therapeutic candidate for NAFLD treatment.

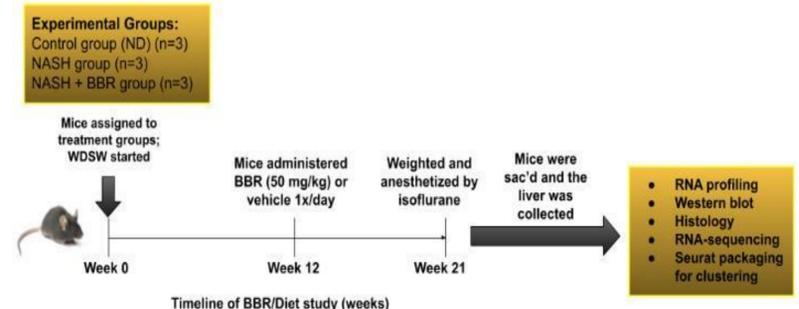
## Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide and has recently been recognized as the fastest-growing cause for liver transplantation due to end-stage liver disease and hepatocellular carcinoma (HCC). The first stage of NAFLD is NAFL- characterized by the development of steatosis where lipids accumulate in over 5% of hepatocytes. While most patients with NAFL have simple steatosis, some go on to develop inflammation and progressive fibrosis. Past studies have identified Berberine, a traditional Chinese medicinal herb, as a potential therapeutic treatment for liver disease. Our previous data shows that BBR interacts by regulates modulates ER stress in macrophages and hepatocytes, to reduce inflammation caused by palmitic acid and the bacterial product lipopolysaccharide (LPS). Based on these data, we hypothesized that Berberine modulates proinflammatory and ER stress pathways to limit hepatic fibrosis, inflammation, and lipid accumulation in the high-fat diet mouse model of NAFLD.

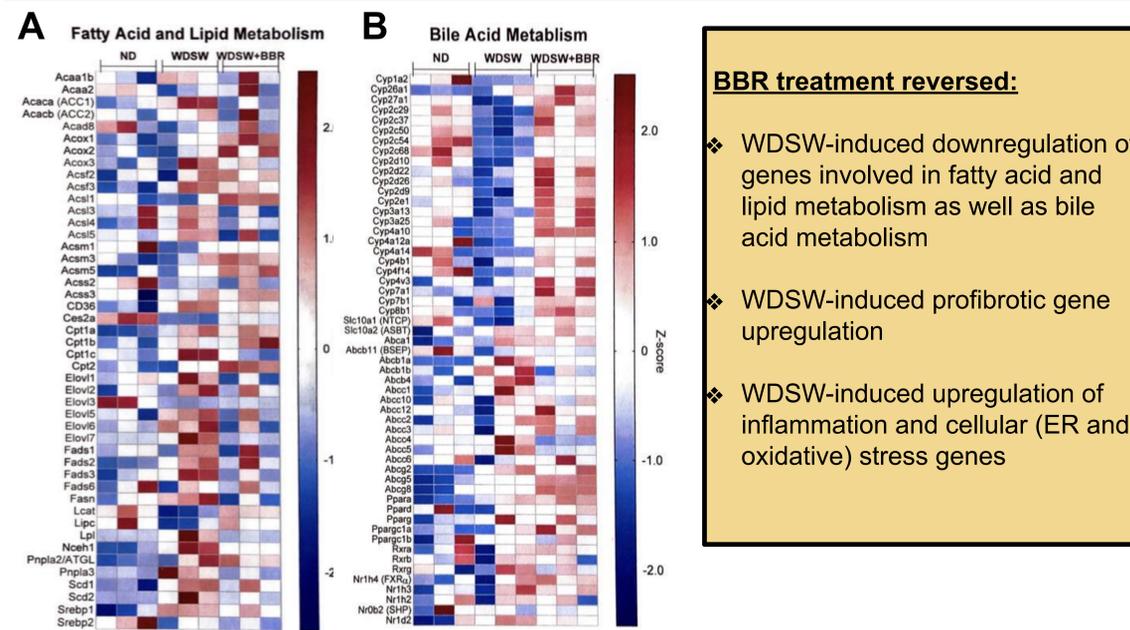


## Methods

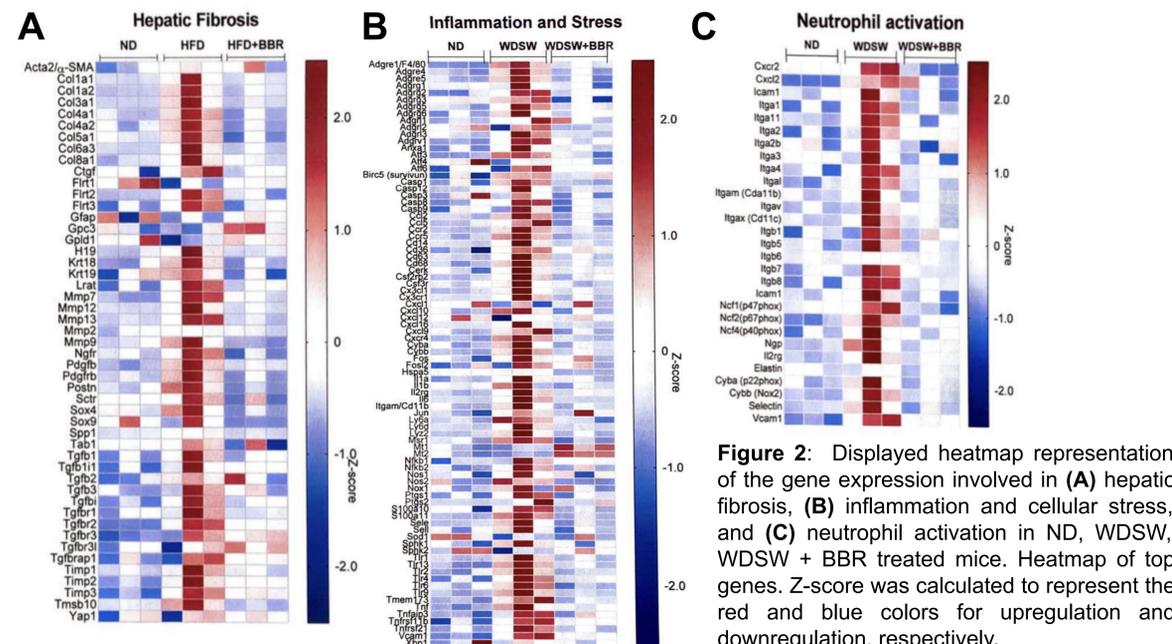
For this study, male and female mice (age 8-12 weeks) were assigned randomly into cohorts: Normal diet (control), NASH group, NASH + BBR group. NASH mice were fed a high-fat and high-carbohydrate diet (42% kcal from fat) with D-fructose (23.1g/L) & D-glucose (18.9g/L) water solution (sweet water; SW) for 21 weeks. Beginning at week 12 of the 21-week feeding timeline, mice in the were treated with BBR (50 µg/g) or vehicle daily via oral gavage. After feeding and treatment, the mice were anesthetized with isoflurane and sacrificed by exsanguination. The whole liver was harvested for histology, RNA profiling, and Western blot analysis. A portion of liver tissue was processed for bulk RNA-sequencing (RNA-seq). After data conversion and normalization, the standard Seurat package was used for clustering, and the differentially expressed genes (DEGs) were calculated.



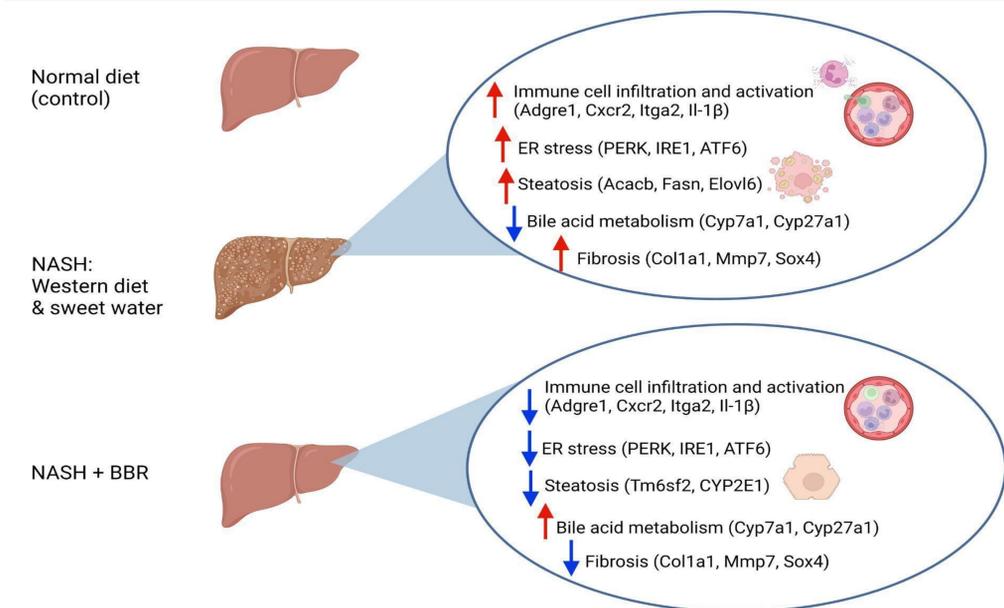
## Results



**Figure 1:** Displayed heatmap representation of the gene expression involved in (A) fatty Acid and Lipid Metabolism and (B) bile acid metabolism of ND, WDSW, WDSW + BBR. Heatmap of top genes. Z-score was calculated to represent the red and blue colors for upregulation and downregulation, respectively.



## Discussion/Conclusion



- Analysis of the heatmaps showed that WDSW feeding induced upregulation of 1035 genes and downregulation of 284 genes, while BBR treatment downregulated 770 genes and upregulated 184 genes.
- BBR displayed comprehensive information regarding its ability to modulate gene expression at the molecular level.
- These findings suggest that BBR could be a potential candidate for therapeutic usage against NASH

## Acknowledgements

I would like to thank Dr. Zhou, Kaitlyn Jackson and the rest of the Zhou lab for their helpful assistance and supportive guidance around the lab.  
I would like to thank Dr. Whitehurst-Cook for sponsoring this wonderful program and the co-directors of the VCU MSIP directors Aditya Kotha, Anaya Surve, and Siddarth Venigalla for their excellent guidance and organization of the program.

## References

Ren S, Ma X, Wang R, Liu H, Wei Y, Wei S, Jing M, Zhao Y. Preclinical Evidence of Berberine on Non-Alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis of Animal Studies. *Frontiers in Pharmacology*, 2021.

Wang Y, Zhou X, Zhao D, Wang X, Gurley EC, Liu R, et al. (2020) Berberine inhibits free fatty acid and LPS-induced inflammation via modulating ER stress response in macrophages and hepatocytes. *PLoS ONE*, 2020.

Wang, Y.; Tai, Y.-L.; Zhao, D.; Zhang, Y.; Yan, J.; Kakiyama, G.; Wang, X.; Gurley, E.C.; Liu, J.; Liu, J.; et al. Berberine Prevents Disease Progression of Nonalcoholic Steatohepatitis through Modulating Multiple Pathways. *Cells*, 2021.