Mannose attenuates liver steatosis through ketohexokinase in a mouse model of NASH

Mount Sinai Kravis Children's Hospital Department of Pediatrics

John G Hong[1], Joshaya Trotman[1], Yvette Carbajal[1], Mariel Glass[1], Peng Zhang[1], Liheng Wang[1], Li Chen[2], Matthieu Petitjean[2], Charles DeRossi[1], Jaime Chu[1]

[1]Icahn School of Medicine at Mount Sinai, New York, New York, USA, [2]Pharmanest Inc, Princeton , NJ, USA

BACKGROUND

- Currently, there are no approved therapies to prevent or reverse the progression of non-alcoholic steatohepatitis (NASH), thus identifying novel therapies is imperative.
- Mannose, a simple sugar and C-2 epimer of glucose, has been long overlooked, but mannose supplementation has recently emerged to have disease-modifying roles in obesity, diabetes, and cancer (Zhou et al. *Frontiers in Immunology* 2021).
- Our group has shown that mannose supplementation can dampen hepatic stellate cells in vitro and dampen fibrogenesis in vivo (DeRossi et al., *Hepatology,* 2019).

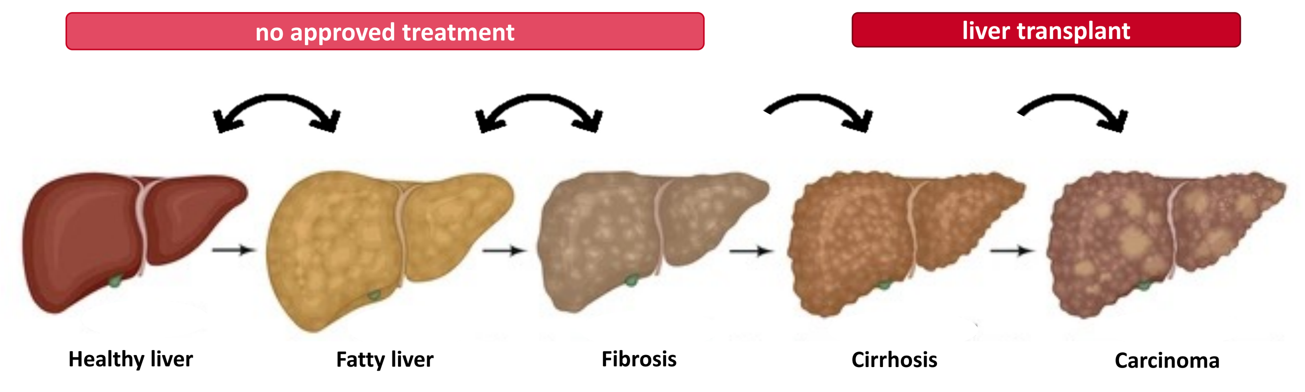

Figure 1. Schematic overview of liver disease progression from healthy liver to cirrhosis.

*From Shutterstock

AIM

Given the individual patient and public health burdens of NAFLD with scarcity of therapeutic options, we sought to determine whether mannose has therapeutic effects in NASH.

METHODS

Mice were fed normal diet (ND) or FAT-NASH regimen (high fat, fructose, cholesterol, and low dose CCl_4) for 12 weeks (Tsuchida et al., *J Hepatol* 2018). Mannose was supplied in drinking water (5% or 20%) for either the full 12 weeks or after 6-week delay (Fig 2). Mouse liver sections were stained with Oil Red O (ORO) to assess steatosis. We used an unbiased, AI-based approach to quantify steatosis phenotypes in liver histological sections (FibroNest^{TM}, Princeton, USA). Liver triglycerides (TG) and cholesterol were measured in all groups. In vitro, primary mouse hepatocytes and human hepatocytes (THLE-5B) were treated with oleic + palmitic acid and fructose for 72 hours. Hepatocytes were treated with 10 and 25mM mannose for 72 hours. ORO staining was used to assess changes in steatosis. Bulk liver RNA-seq was performed on whole mouse NASH livers with and without mannose treatment (Genewiz). Western blots and qPCR were performed on mouse livers and human hepatocytes.

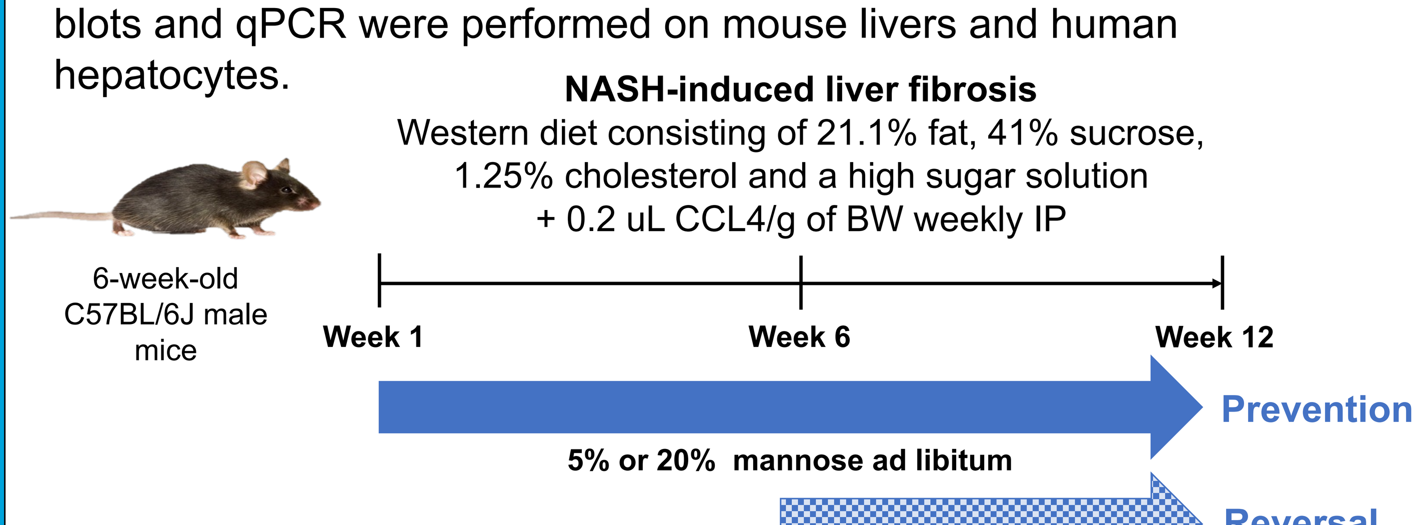

Figure 2. Schematic overview of the FAT-NASH mouse model and mannose

RESULTS

I. MANNOSE ATTENUATES STEATOSIS, LIVER TRIGLYCERIDES AND CHOLESTEROL IN A NASH MOUSE MODEL

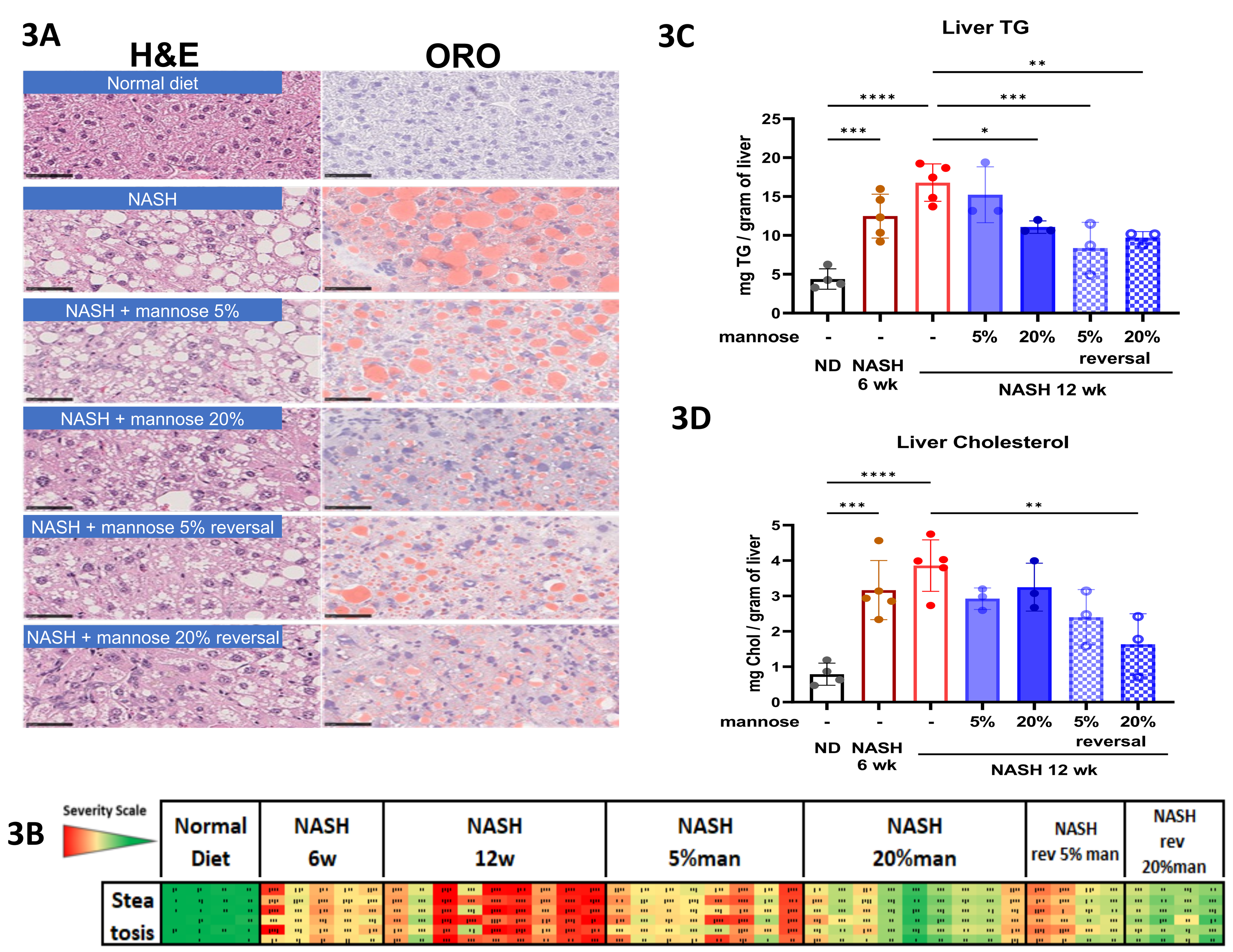

Figure 3. A. Mouse liver sections stained with H&E (left panels) and ORO (right panels) with and without mannose treatments. **B.** AI-approach to histologic assessment revealed mannose supplementation improved steatosis at 5% and 20% (-26% and -60%, p<0.05) with prophylactic and delayed treatment in NASH mice, *in vivo,* at 12 weeks. **C.** Mannose treatment reduced liver TG in all NASH mice, particularly with delayed 6-week treatment of mannose 5 and 20% (p<0.001 and <0.01 respectively, n=3-5). **D.** Liver cholesterol was also significantly reduced in the reversal group treated with 20% mannose after 6-week delay (p<0.01, n=3-5). *p<0.05, **< 0.01, *** < 0.001

II. MANNOSE REDUCES STEATOSIS *IN VITRO*

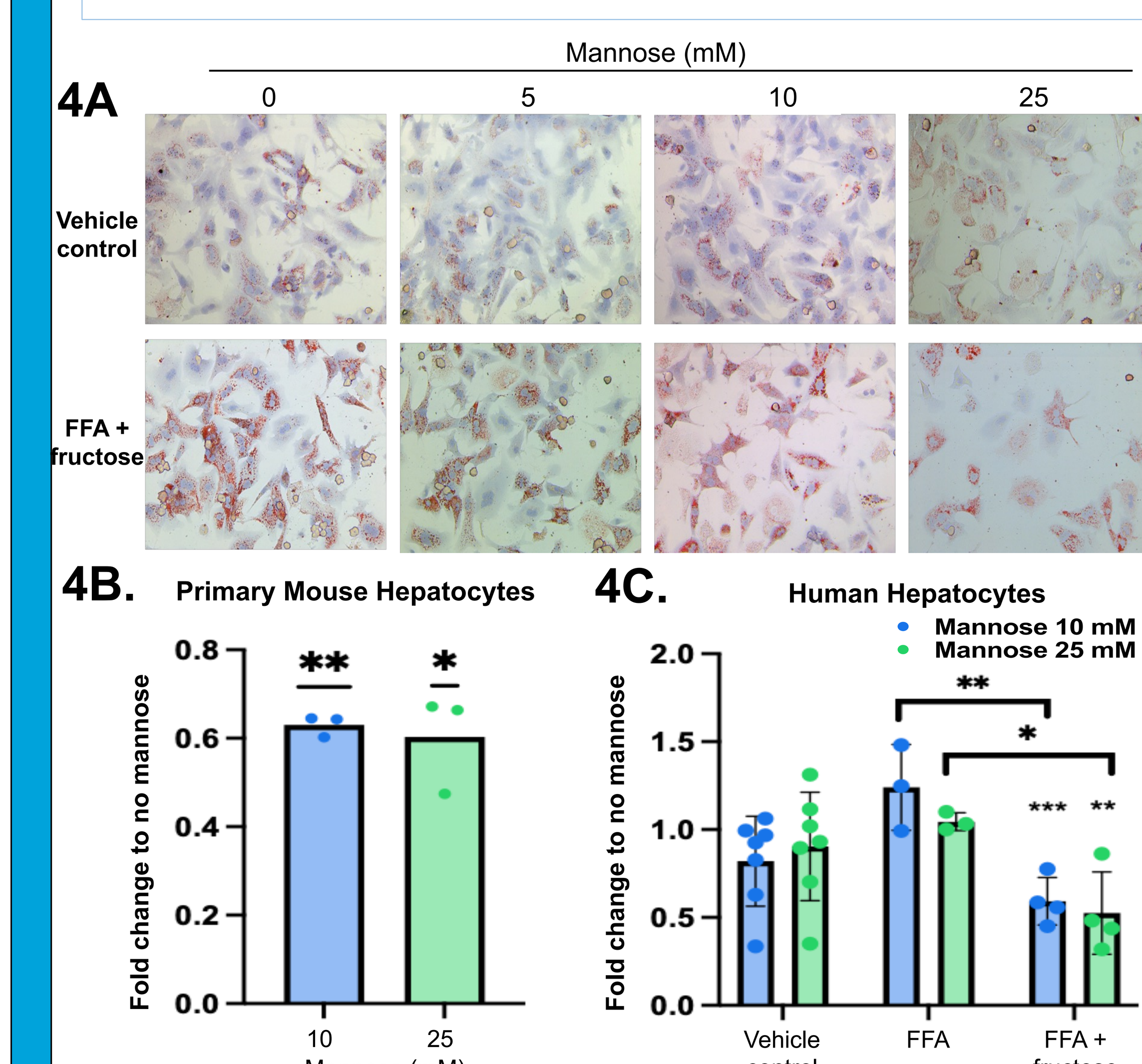

Figure 4. A. ORO staining of primary mouse hepatocytes with and without mannose. **B.** Mannose treatments at 10 and 25 mM reduced steatosis by 40% (p<0.01 and p<0.05 respectively, n=3) in primary mouse hepatocytes. **C.** Mannose reduced steatosis by 33% at 10 and 25 mM (p<0.001 and p<0.01 respectively, n=3-4) in human hepatocytes (THLE-5B). Steatosis reduction with mannose treatment was only seen in fructose-conditioned hepatocytes and not in FFA alone. *p<0.05, **< 0.01, *** < 0.001.

III. MANNOSE SUPPLEMENTATION DAMPENS FRUCTOSE UPTAKE AND METABOLISM GENES IN MOUSE NASH LIVERS

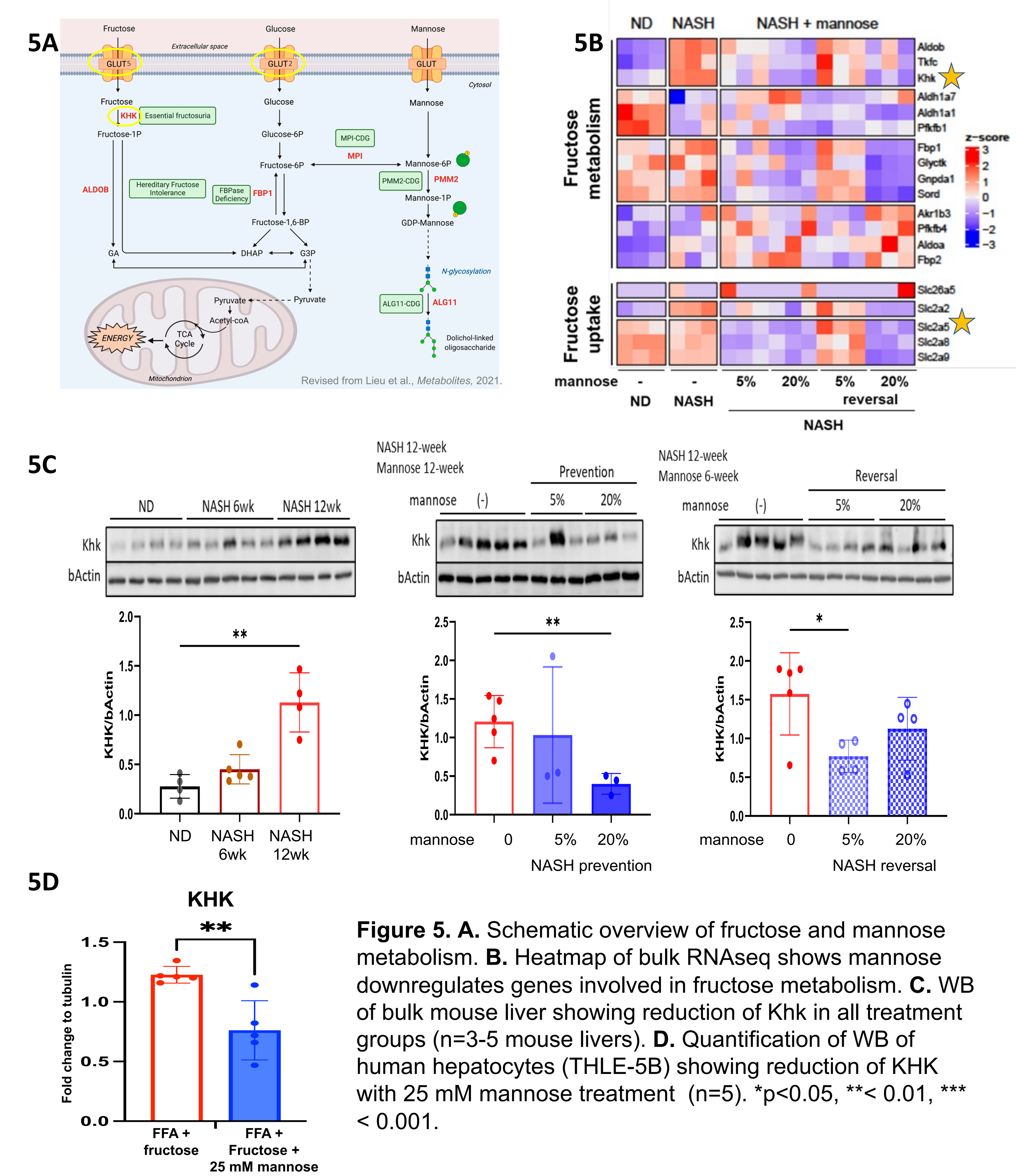

Figure 5. A. Schematic overview of fructose and mannose metabolism. **B.** Heatmap of bulk RNASeq shows mannose downregulates genes involved in fructose metabolism. **C.** WB of bulk mouse liver showing reduction of Khk in all treatment groups (n=3-5 mouse livers). **D.** Quantification of WB of human hepatocytes (THLE-5B) showing reduction of KHK with 25 mM mannose treatment (n=5). *p<0.05, **< 0.01, *** < 0.001.

CONCLUSIONS

- Mannose reduces steatosis *in vivo* marked by ORO staining, AI-guided analysis, and liver TG and cholesterol measurements.
- Mannose attenuates steatosis *in vitro* in primary mouse and human hepatocytes and anti-steatotic effect of mannose is dependent on fructose conditioning.
- Mannose supplementation dampens fructose uptake and metabolism genes. Ketohexokinase (KHK), the main enzyme involved in fructolysis, is dampened and is of particular interest.
- Ultimately, our findings uncover mannose as a novel and potential NAFLD therapy. Ongoing studies will test the role of mannose and KHK in liver steatosis.

Funding sources: R01DK121154, R01DK121154-03S1 (JC) and the PKD Foundation Young Investigators Award (CD). Drs. Chen and Petitjean are part of Pharmanest and Dr. Chu is a consultatnt to Albireo.