KEYSTONE SYMPOSIA

Metabolic and Molecular Mechanisms of NAFLD/NASH
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Pathology Image Analysis Accurately Quantifies the Anti-Fibrotic and Anti-Steatotic effects of mannose in Rodent NASH Model

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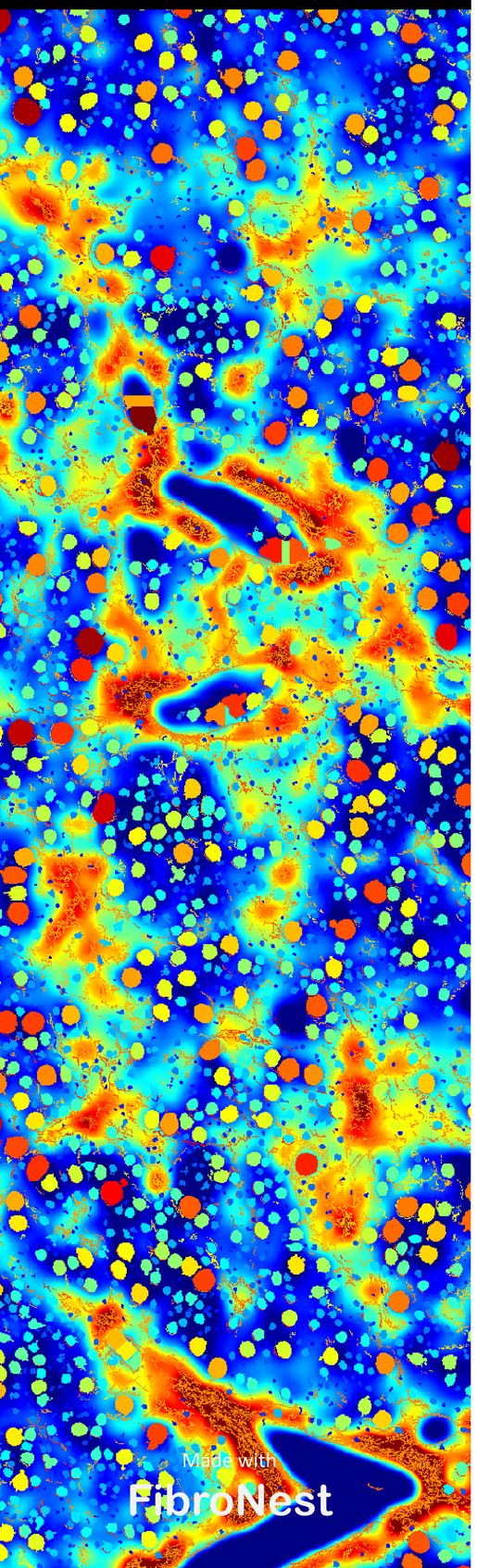


1 Introduction

The defining pathologic elements of non-alcoholic steatohepatitis (NASH) are fat accumulation, inflammation, and fibrosis. While conventional histopathology remains the gold standard for diagnosis and staging, this method has limitations, as it uses a narrow range for scoring, qualitative evaluation, and it is also prone to inter-observer variability and categorical staging uncertainty.

2 Aim

Here, we used automated single-fiber, single vacuole quantitative Image analysis (FibroNest™, Princeton, USA) to quantify the changes of the fibrosis and steatosis phenotypes. We have previously shown the anti-fibrotic effects of mannose in human hepatic stellate cells in vitro (DeRossi et al., Hepatology 2019; doi: 10.1002/hep.30677), and apply FibroNest to evaluate the prophylactic and therapeutic effects of mannose administration (low and high doses) in the FAT-NASH model (Tsuchida et al, J. Hepatology 2018; doi: 10.1016/j.jhep.2018.03.011).

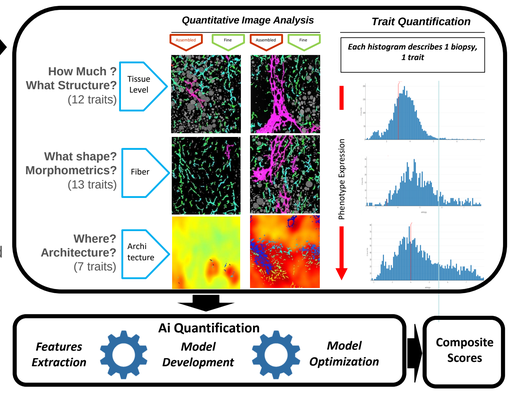
3 Method

Mice were fed a normal diet or the FAT-NASH regimen (high fat, high fructose, high cholesterol, and a very low dose CCl4) for 6 and 12 weeks to induce NASH (NASH-6w, n=5 | NASH-12w, n=9).

Two groups of mice received prophylactic administration of mannose (D-mannose, a 2-epimer of glucose) at the beginning of the 12 weeks NASH diet at low (5% mannose in the drinking water, NASH-5%man group, n=8) and high (20% mannose in the drinking water, NASH-20%man group, n=9).

In addition, animals treated for 12w of NASH diet received therapeutic mannose administration starting at week six with either low dose (5% mannose), NASH-rev 5% man group (n=4) or high dose (20% mannose, NASH-rev 20% man group, n=4).

Liver histological sections were stained with picrosirius red for collagens and imaged at 40X in white light with an Aperio Digital Pathology system.

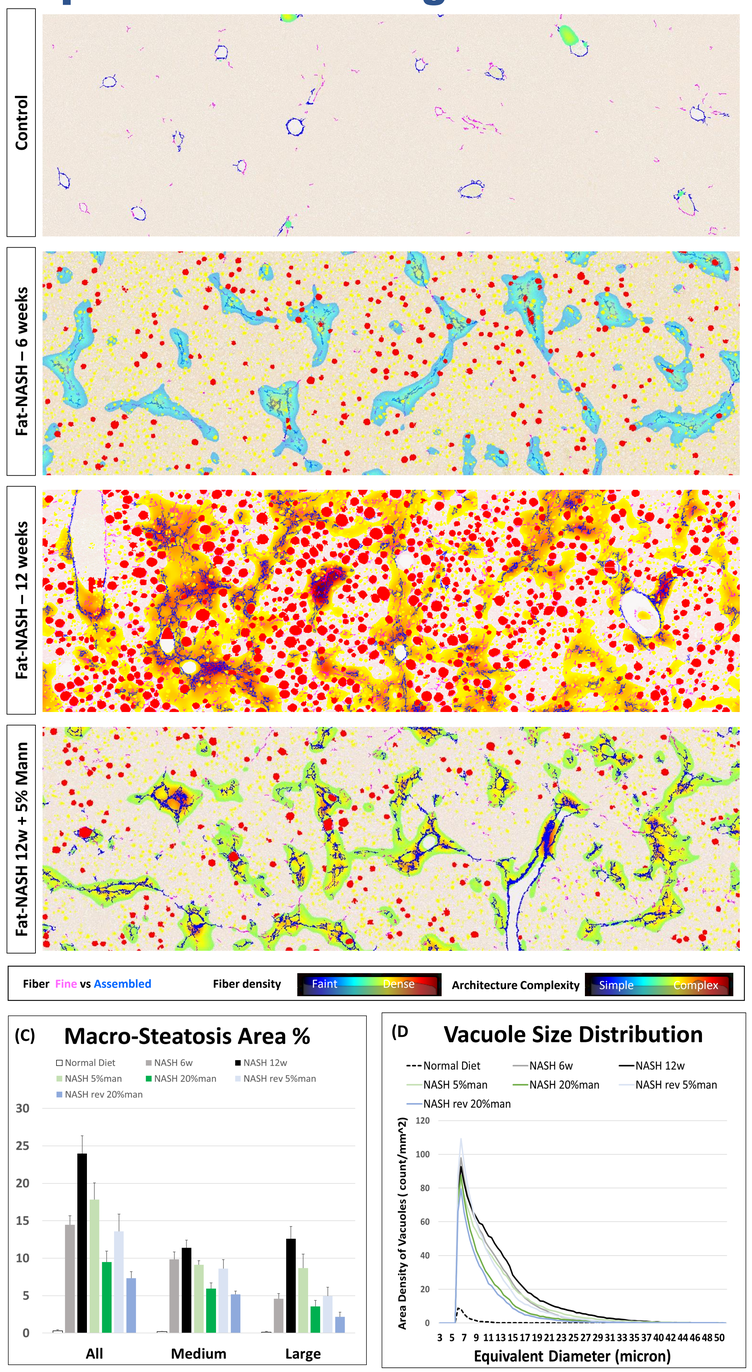


FibroNest™, a cloud-based image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for collagen content and structure, fiber morphometry, and architecture (measures the organization of the fibers).

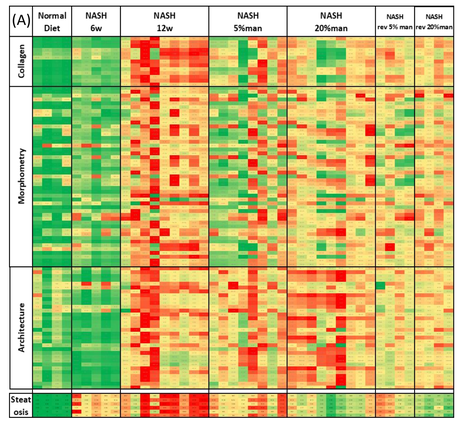
Principal quantitative fibrosis traits (up to 315 qFTs) are automatically detected and combined into a Phenotypic Composite Fibrosis Score (PT-Ph-FCS) that is normalized to the parenchymal area (excluding the steatotic area).

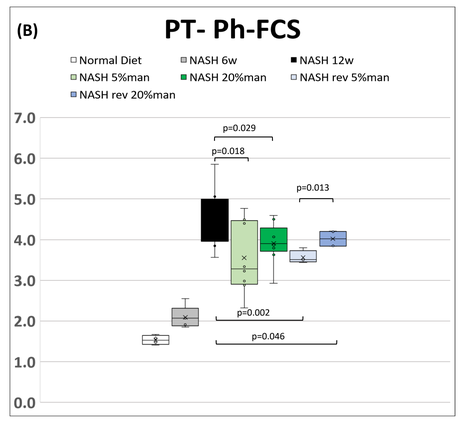
Steatotic hepatocytes were identified to quantify macro-Steatosis Area (%) using Medium fat vacuoles (6mm<diameter<18mm) and Large vacuoles (diameter>18mm).

4 Representative Images



Results





The Fat-NASH regimen induces NASH at 6 weeks and peaked at 12 weeks with increases in PT-Ph-FCS (Fig.B) and with increases in Steatosis Area (Fig. C), as compared to Normal Diet. Phenotypic heat-charts (Fig. A) recapitulate the changes in the phenotype of Fibrosis.

NASH (12wk) -induced Fibrosis and Steatosis are reduced with prophylactic treatment of 5% and 20% mannose for PT-Ph-FCS (-23% and -15%, respectively) and for Steatosis Area (-26% and -60%, respectively). Similar reduction of NASH (12wk) is also seen with therapeutic treatment of 5% and 20% mannose for PT-Ph-FCS (-23% and -13%) and for Steatosis Area (-43% and -70%, respectively).

The steatotic effects are mostly driven by the large hepatic fat vacuoles (Fig. D)

5 Conclusions

In this study, we showed that mannose has both anti-steatotic and anti-fibrotic effects during prophylactic and therapeutic treatments for Nonalcoholic Steatohepatitis. FibroNestTM provides robust quantitative digital pathology measurements of fibrosis (and steatosis) severity and regression in NASH for the assessment of compounds or pharmacological agents.

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