Profiling fibrosis regression in a rat model of non-alcoholic steatohepatitis

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Introduction

- Severe liver fibrosis and cirrhosis increase the risk of liver-related and all-cause mortality in patients with non-alcoholic steatohepatitis (NASH).[1]
- There is increasing evidence that liver fibrosis, regardless of etiology, is reversible.[2]
- Accurately quantifying fibrosis regression in the clinical setting remains challenging.
- The number of investigational agents that directly target fibrosis currently under evaluation for the treatment of NASH remains low.

Aim

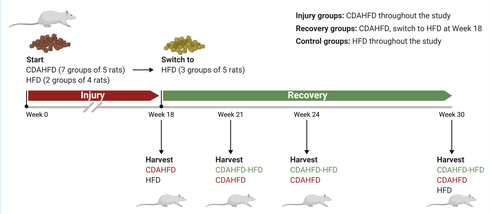
- The aim of this study was to establish a rodent model of fibrosis progression and regression in the context of NASH to profile the molecular pathways and biomarkers driving fibrosis.

Methods

- A total of 9 groups of male Wistar rats (6 weeks of age) were assigned to receive either a choline-deficient, L-amino acid-defined, high-fat (60 kcal %) diet (CDAHFD; 7 groups of 5 rats) or a control, high-fat diet (HFD; 2 groups of 4 rats) ad libitum for 18 weeks (**Figure 1**).
- At 18 weeks, 3 groups receiving a CDAHFD were switched to receive a HFD for an additional 3, 6, or 12 weeks (specified as 'recovery' groups, n=5 per time point), and 3 groups continued on a CDAHFD for an additional 3, 6, or 12 weeks (specified as 'injury' groups, n=5 per time point).
  - The control groups comprised rats that received a HFD throughout the study, until harvest (n=4 per time point).
- To enable the measurement of collagen fractional synthesis rate, all rats received ^2H_2O for the 3 weeks preceding harvest.
- Rats were sacrificed at designated time points (**Figure 1**). Liver tissue and blood samples were collected for the evaluation of liver injury and recovery, by determining:
  - Liver enzyme levels (ALT [alanine transaminase], AST [aspartate transaminase], and ALP [alkaline phosphatase]; performed by IDEXX Laboratories).
  - Fibrogenic gene expression (*collagen Type I alpha 1* [*Col1a1*]; performed using the NanoString platform).
  - Collagen fractional synthesis rate (performed by Metabolic Solutions, Inc.).
  - Tissue injury, as assessed by histology (including myofibroblast expansion, as determined by alpha smooth-muscle actin [αSMA] immunohistochemistry, and collagen accumulation, as determined by picrosirius red [PSR] staining; performed by Acepix Biosciences, Inc.).

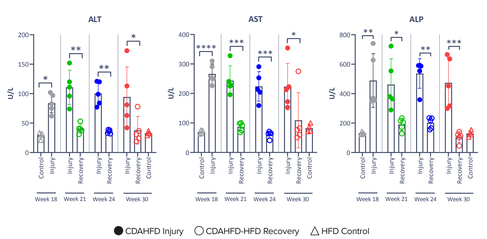
Figures

Figure 1. NASH recovery model



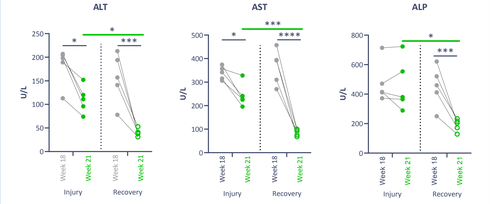
CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; HFD, high-fat diet; NASH, non-alcoholic steatohepatitis

Figure 2. Liver enzyme levels were reduced with recovery



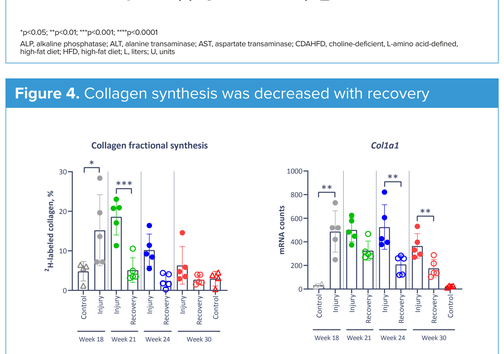
*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; HFD, high-fat diet; L, liters; U, units

Figure 3. Liver enzymes in individual rats were reduced with recovery



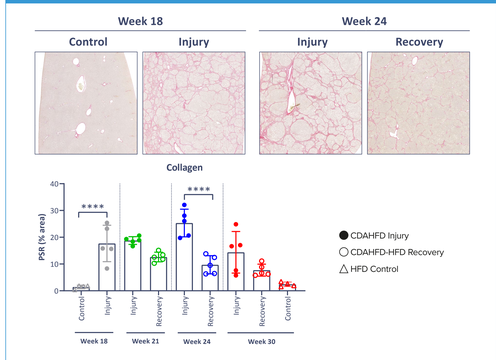
*p<0.05; ***p<0.001; ****p<0.0001
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; L, liters; U, units

Figure 4. Collagen synthesis was decreased with recovery



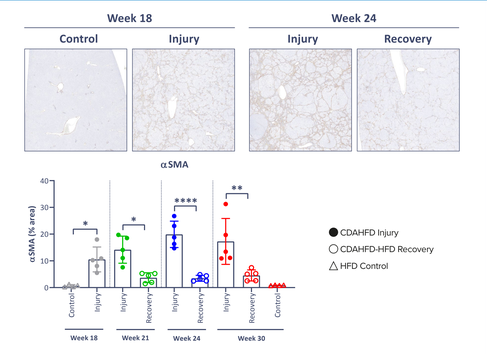
*p<0.05; **p<0.01; ***p<0.001
CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; *Col1a1, collagen Type I alpha 1*; HFD, high-fat diet; mRNA, messenger ribonucleic acid

Figure 5. Collagen accumulation was decreased with recovery



****p<0.0001
CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; HFD, high-fat diet; PSR, picrosirius red

Figure 6. Myofibroblasts were decreased with recovery



*p<0.05; **p<0.01; ***p<0.0001
αSMA, alpha smooth-muscle actin; CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; HFD, high-fat diet

Results

- Rats within the injury groups (i.e., rats receiving a CDAHFD) had elevated levels of liver enzymes, compared with rats in the recovery groups (i.e., rats that switched to a HFD after receiving a CDAHFD) and rats in the control groups (i.e., rats receiving a HFD; **Figure 2**).
  - Switching to a HFD at Week 18 resulted in significant decreases (>50%) in liver enzyme levels, compared with rats that remained on a CDAHFD (p<0.05).
- Within each individual rat, liver enzyme levels were measured after 18 weeks of injury and at the time of harvest (shown for Week 21 in **Figure 3**).
  - While the levels of ALT and AST significantly decreased in all rats from Week 18 to Week 21 (p<0.05), the most consistent and significant reductions in ALT, AST, and ALP levels were observed in rats in the recovery groups (p<0.001).
- Rats in the injury groups had increases in collagen fractional synthesis rates and *Col1a1* gene expression (**Figure 4**).
  - Switching to a HFD resulted in a significant decrease in collagen fractional synthesis rate after 3 weeks (an approximately 70% decrease in the percentage of ^{2}H-labelled collagen; p<0.001) and collagen gene expression after 6 weeks (an approximately 60% decrease in *Col1a1* gene expression; p<0.01), compared with rats that remained on a CDAHFD.
- Rats in the injury groups had increased levels of liver fibrosis, as determined by collagen accumulation (measured by the percentage of PSR staining; **Figure 5**).
  - Switching to a HFD resulted in a significant decrease in collagen accumulation after 6 weeks (an approximately 60% decrease in the percentage of PSR area), compared with rats that remained on a CDAHFD (p<0.0001).
- Rats in the injury groups had an increased number of myofibroblasts within liver sections, as determined by αSMA immunohistochemistry (**Figure 6**).
  - Switching to a HFD resulted in a significant decrease in myofibroblasts after 3 weeks (an approximately 75% decrease in the percentage of αSMA area), compared with rats that remained on a CDAHFD (p<0.05).

Conclusions

- **The data support the establishment of a rodent model of fibrosis progression and regression, facilitating accurate quantification of fibrosis regression in the context of NASH.**
  - **A CDAHFD resulted in predictable progression of fibrosis in rats.**
  - **Switching to a HFD resulted in consistent regression of fibrosis that can be quantified.**
- **This model will be useful to evaluate the pathways and drug targets driving fibrogenesis and regression and to identify biomarkers of these processes that may be used to monitor fibrosis regression in a clinical setting.**

References: 1. Dulai PS, Singh S, Patel J, et al. *Hepatology* 2017;65(5):1557–1565;
2. Bataller R, Brenner DA. *J Clin Invest* 2005;115(2):209–218

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Miscellaneous: Poster 0321 presented at The Liver Meeting Digital Experience™ (TLMdX), American Association for the Study of Liver Diseases (AASLD), 13–16 November 2020.

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