# Disease progression in the non-obese CDAA-HFD mouse model of advanced NASH and fibrosis


**Authors**
Marco Tozzi, Jacob Nøhr-Meldgaard, Ditte D. Thorbeck, Denise Oro, Martin Rønn Madsen, Henrik H. Hansen, Michael Feigh.

Gubra, Hørsholm, Denmark

**Corresponding author**
Michael Feigh - mfe@gubra.dk



## BACKGROUND & AIM

Patients with non-alcoholic steatohepatitis (NASH) are at increased risk of developing hepatic fibrosis which can ultimately progress to cirrhosis, hepatocellular carcinoma and end-stage liver disease.

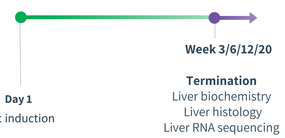
Animal models of NASH with progressive, advanced fibrosis are therefore highly warranted for exploring novel pharmacological treatments.

The present study aimed to in-depth characterize disease progression of the non-obese Choline-Deficient l-Amino-Acid defined High-Fat Diet (CDAA-HFD) mouse model of NASH and fibrosis.

## METHODS

C57BL/6JRj mice were fed chow or CDAA-HFD (45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 3, 6, 12 or 20 weeks before termination (n=11-12 per group). Chow-fed mice (n=8) served as normal controls. Terminal endpoints included plasma and liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage, quantitative liver histology and transcriptome signatures.


## 1 Study outline




| Group no. | Group | Name | Number of animals |
|---|---|---|---|
| 1 | Chow | Chow | 8 |
| 2 | CDAA-HFD 3W | 3w | 12 |
| 3 | CDAA-HFD 6W | 6w | 12 |
| 4 | CDAA-HFD 12W | 12w | 11 |
| 5 | CDAA-HFD 20W | 20w | 12 |

## 2 Metabolic and biochemical parameters

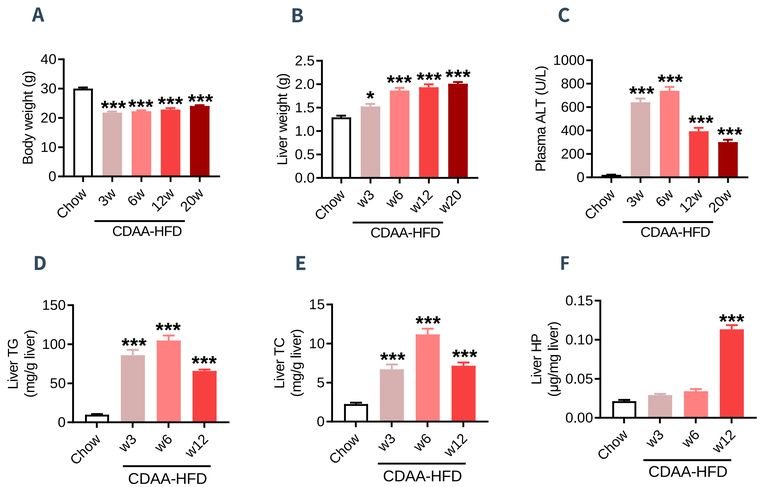



**Figure 1. CDAA-HFD mice are lean, develop hepatomegaly and show increased plasma/liver biomarkers of NASH and fibrosis.** **(A)** Terminal body weight (g). **(B)** Terminal liver weight (g). **(C)** Plasma alanine transaminase (ALT, U/L). **(D)** Liver triglycerides (TG, mg/g liver). **(E)** Liver total cholesterol (TC, mg/g liver). **(F)** Liver hydroxyproline (HP, µg/mg liver). * p< 0.05, ***p<0.001 compared to corresponding Chow control (Dunnett's test one-factor linear model).

## 3 NAFLD Activity Score and Fibrosis Stage

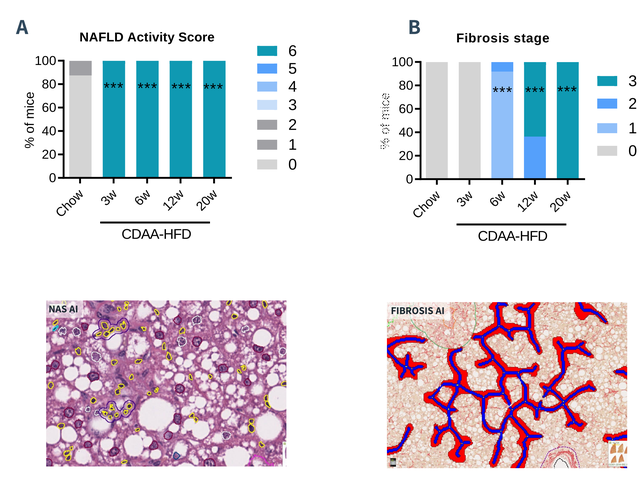



**Figure 2. CDAA-HFD mice demonstrate rapid induction of severe NASH and fibrosis.** Histopathological scores and histomorphometry were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Score. **Bottom panels:** Representative HE and PSR photomicrographs used for GHOST evaluation. ***p<0.001 compared to chow group (One-sided Fisher's exact test with Bonferroni correction).

## 4 Quantitative histological markers of steatosis, inflammation and fibrosis

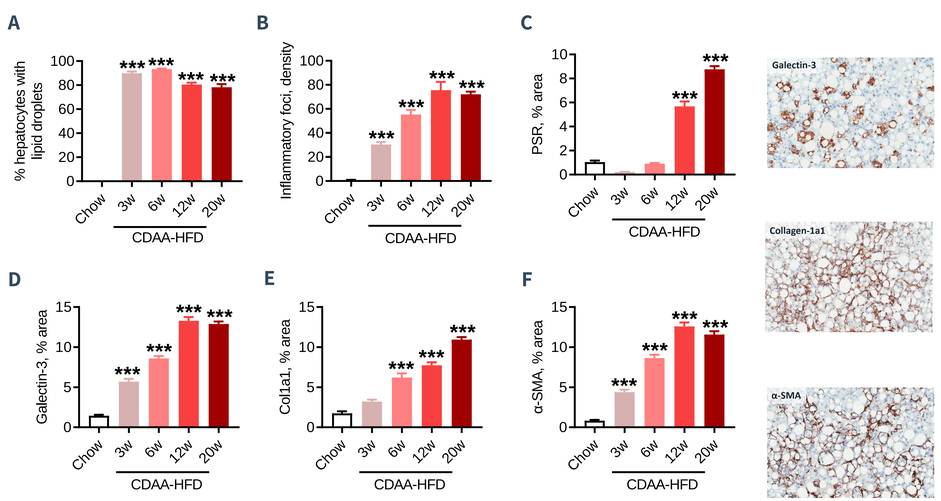



**Figure 3. CDAA-HFD induction progressively increases quantitative liver histological markers for fibrosis and inflammation**.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1 (Col1a1). **(F)** % area of alpha-smooth muscle actin (α-SMA, ~~)as~~-marker for stellate cell activation). Mean ± SEM. ***p<0.001 compared to chow group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for CDAA-HFD 8 weeks (scale bar, 100 µm).

## 5 Liver transcriptome profile

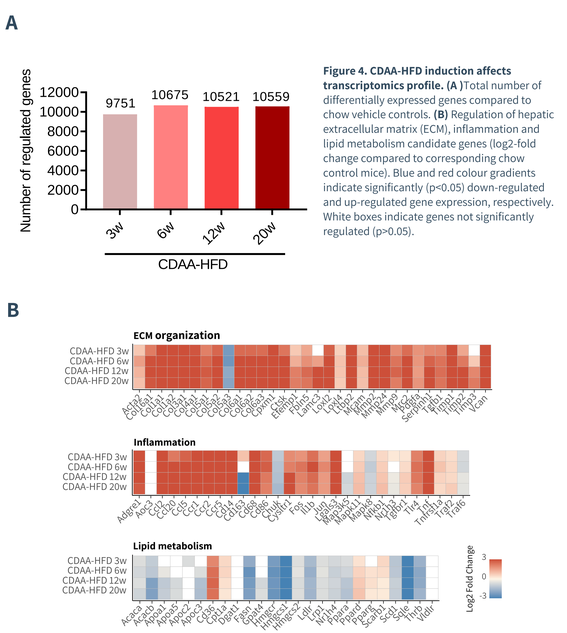



**Figure 4. CDAA-HFD induction affects transcriptomics profile.** **(A )**Total number of differentially expressed genes compared to chow vehicle controls. **(B)** Regulation of hepatic extracellular matrix (ECM), inflammation and lipid metabolism candidate genes (log2-fold change compared to corresponding chow control mice). Blue and red colour gradients indicate significantly (p<0.05) down-regulated and up-regulated gene expression, respectively. White boxes indicate genes not significantly regulated (p>0.05).

## CONCLUSION

The CDAA-HFD mouse:

+ Shows a lean phenotype with hepatomegaly and increased plasma/liver biochemical markers of NASH and fibrosis
+ Demonstrates substantial increases in NAS within 3 weeks of diet-induction
+ Shows progressive increases in liver fibrosis stage from 6 weeks of diet-induction.
+ Develops advanced fibrosis (stage F3) after 12 weeks of diet-induction
+ Quantitative histology supports progressive increases in steatosis, inflammation and fibrosis
+ Rapidly and profoundly influences hepatic gene expression markers of lipid metabolism, inflammation and fibrosis

CDAA-HFD mouse model of advanced NASH and progressive fibrosis allows for profiling anti-fibrotic drugs in prophylactic and therapeutic intervention settings.

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