

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

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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/6J 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

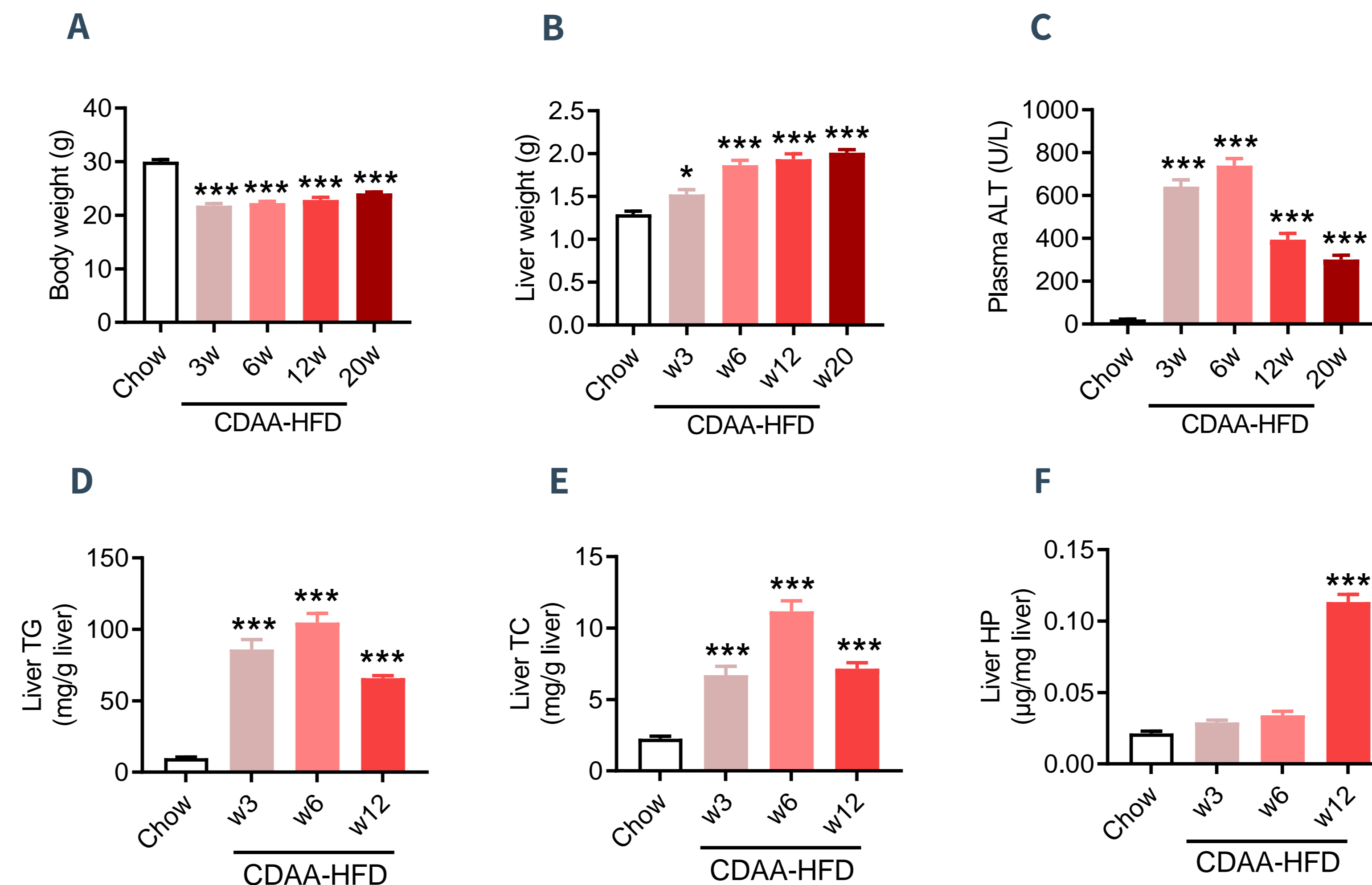


Figure 1. CDAA-HFD mice are lean, develop hepatomegaly and show increased plasma/liver biomarkers of NASH and fibrosis. (A) Terminal body weight (g). (B) Terminal liver weight (g). (C) Plasma alanine transaminase (ALT, U/L). (D) Liver triglycerides (TG, mg/g liver). (E) Liver total cholesterol (TC, mg/g 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

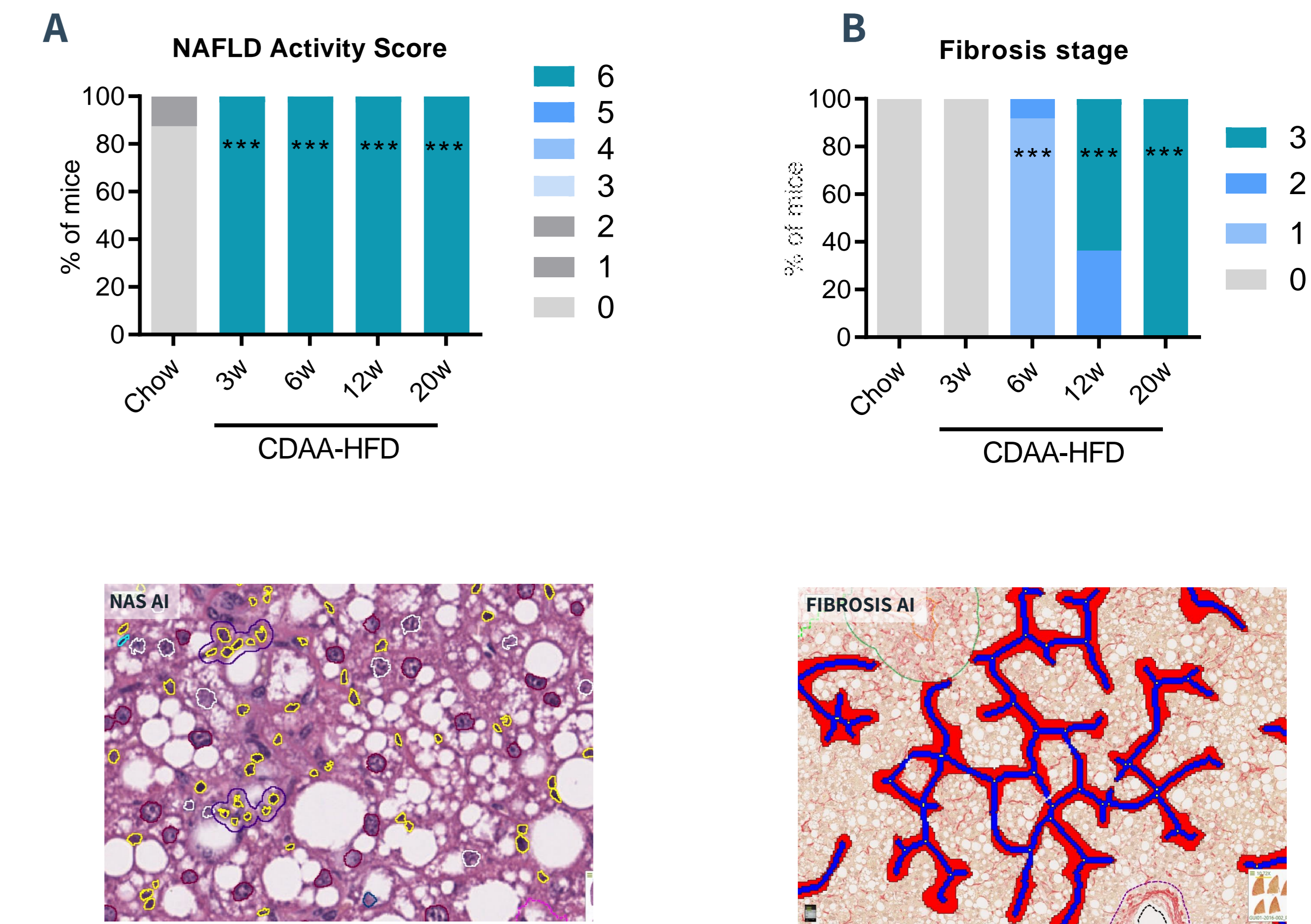


Figure 2. CDAA-HFD mice demonstrate rapid induction of severe NASH and fibrosis. Histopathological scores and histomorphometry were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. (A) NAFLD Activity Score (NAS). (B) Fibrosis Score. Bottom 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

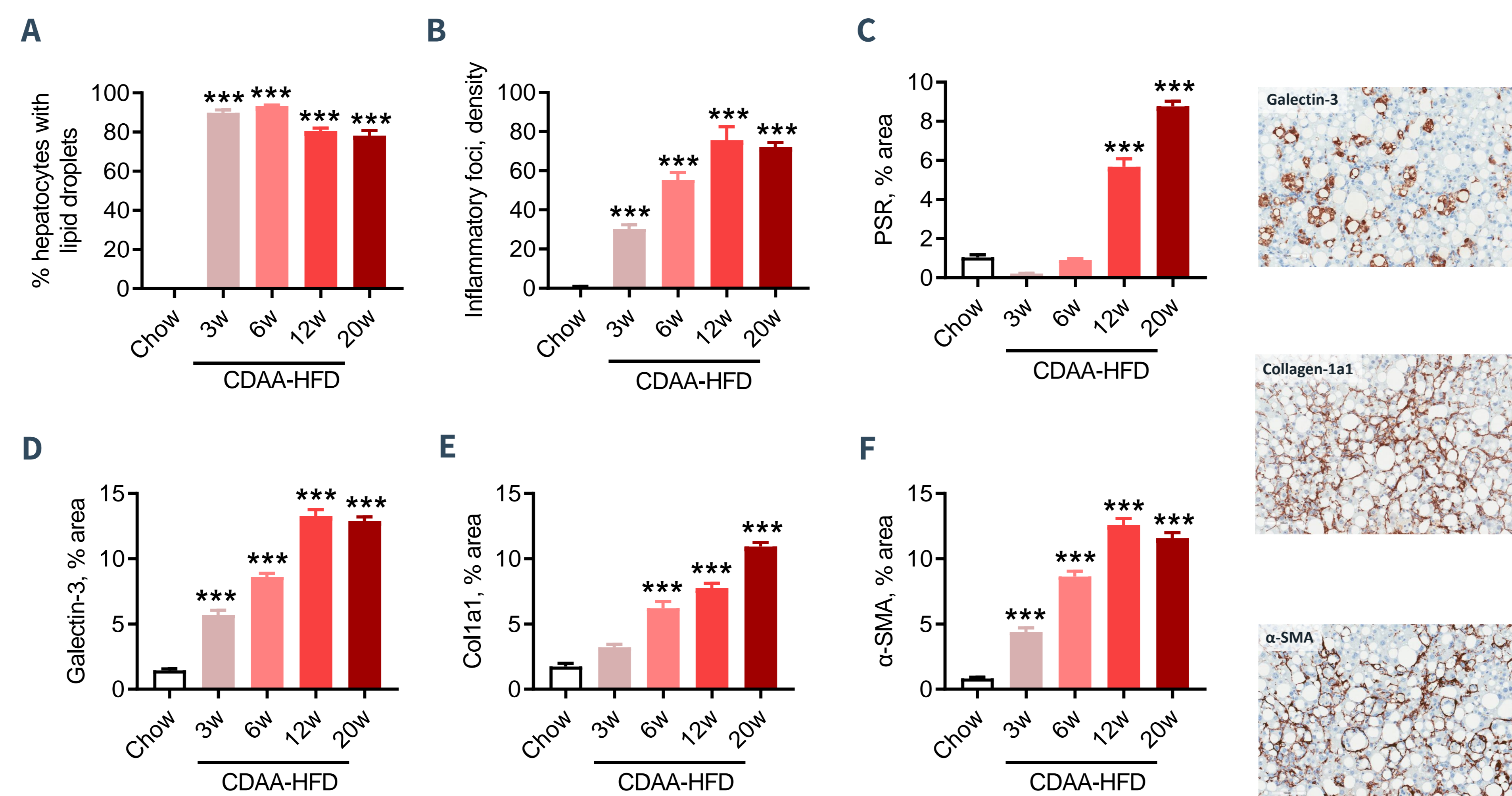


Figure 3. CDAA-HFD induction progressively increases quantitative liver histological markers for fibrosis and inflammation. Histomorphometric assessments were performed by GHOST deep learning-based image analysis for scoring-associated variables (panels A-B) and conventional IHC image analysis (panels 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

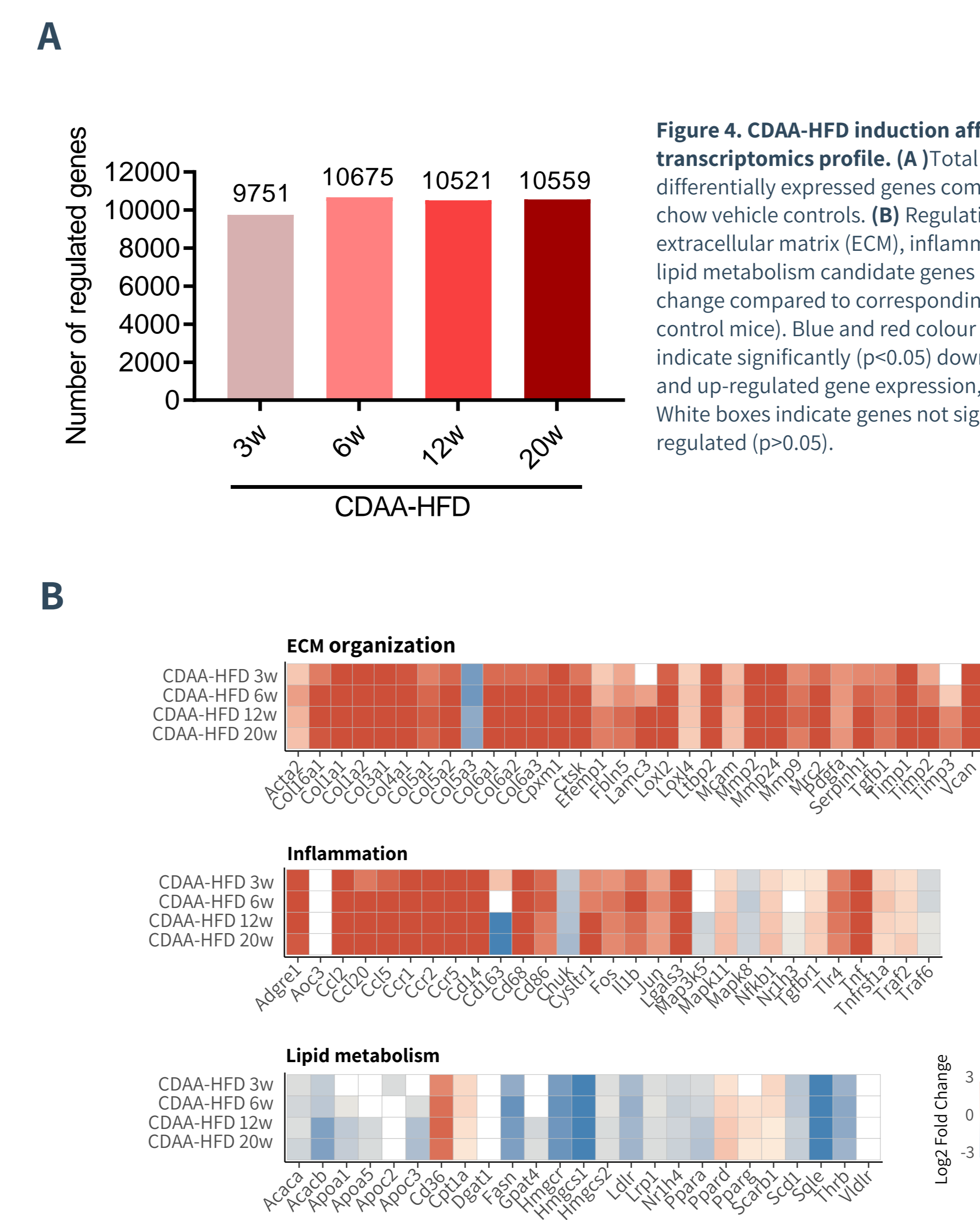


Figure 4. CDAA-HFD induction affects transcriptomics profile. (A) Total number of differentially expressed genes compared to chow vehicle controls. (B) Regulation of hepatic extracellular matrix (ECM), inflammation and lipid metabolism candidate genes (log2-fold change compared to corresponding chow 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.