Metabolic, biochemical and histological characterization of a CDAA-HFD-induced non-obese rat model of advanced NASH with progressive fibrosis

Authors
Malte Hasle Nielsen, Denise Oro, Michael Feigh.

Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

BACKGROUND & AIM

Non-alcoholic steatohepatitis (NASH) predisposes to development of advanced fibrosis and cirrhosis.

Thus, animal models of advanced NASH with fibrotic disease progression is highly warranted for exploring novel pharmacological treatments.

The present study aimed to characterize the metabolic, biochemical and histological effect of Choline-Deficient l-Amino-Acid defined High-Fat Diet (CDAA-HFD) in male Sprague Dawley rats during 12 weeks of dieting.

1 Study outline

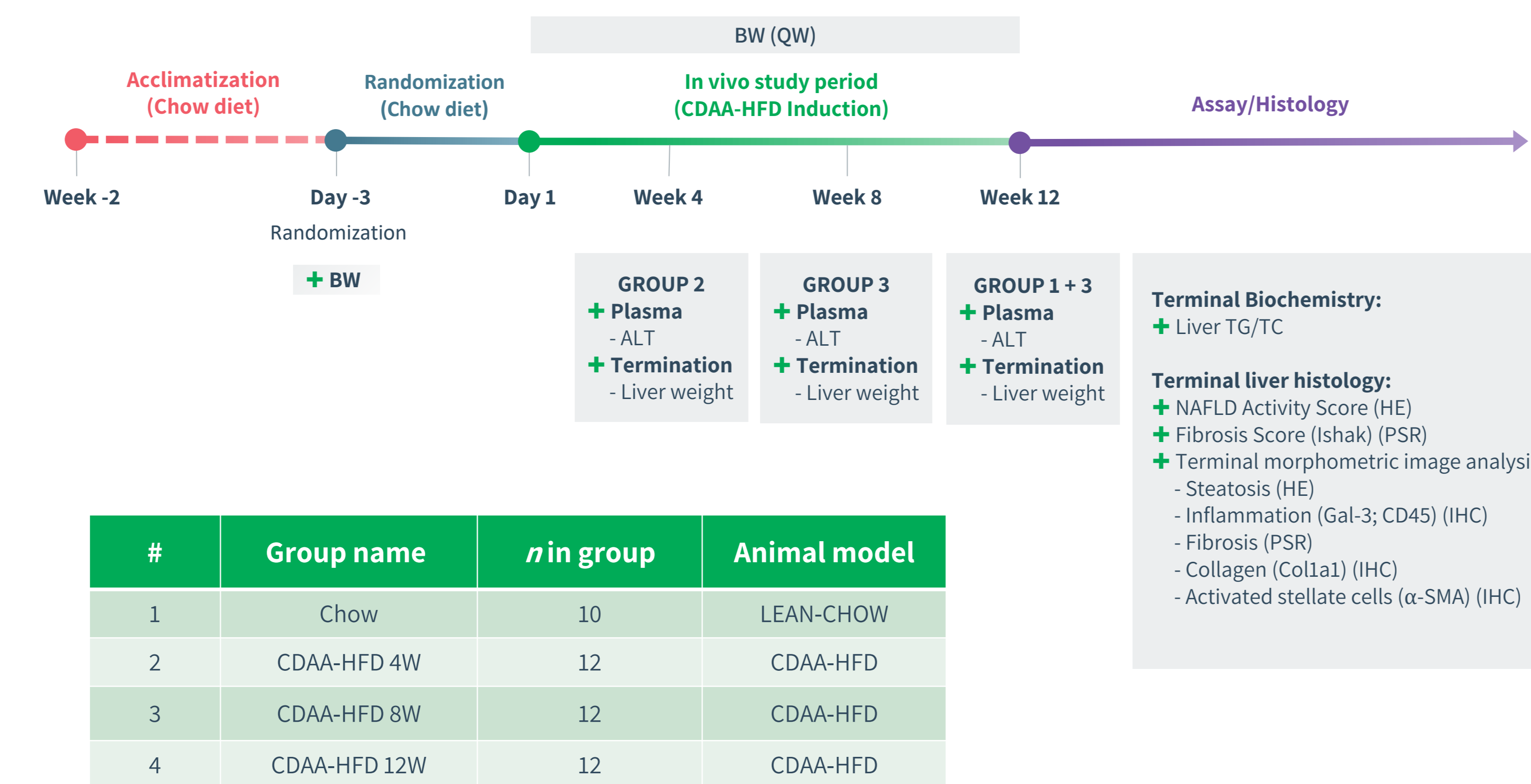| #	Group name	n in group	Animal model
1	Chow	10	LEAN-CHOW
2	CDAA-HFD 4W	12	CDAA-HFD
3	CDAA-HFD 8W	12	CDAA-HFD
4	CDAA-HFD 12W	12	CDAA-HFD

Figure 1. Study outline, groups and intervention.
BW: body weight; QW: once weekly; ALT: alanine aminotransferase; TG: total triglycerides; TC total cholesterol; HE: Haematoxylin Eosin; PSR: Picro Sirius Red; IHC: Immunohistochemistry

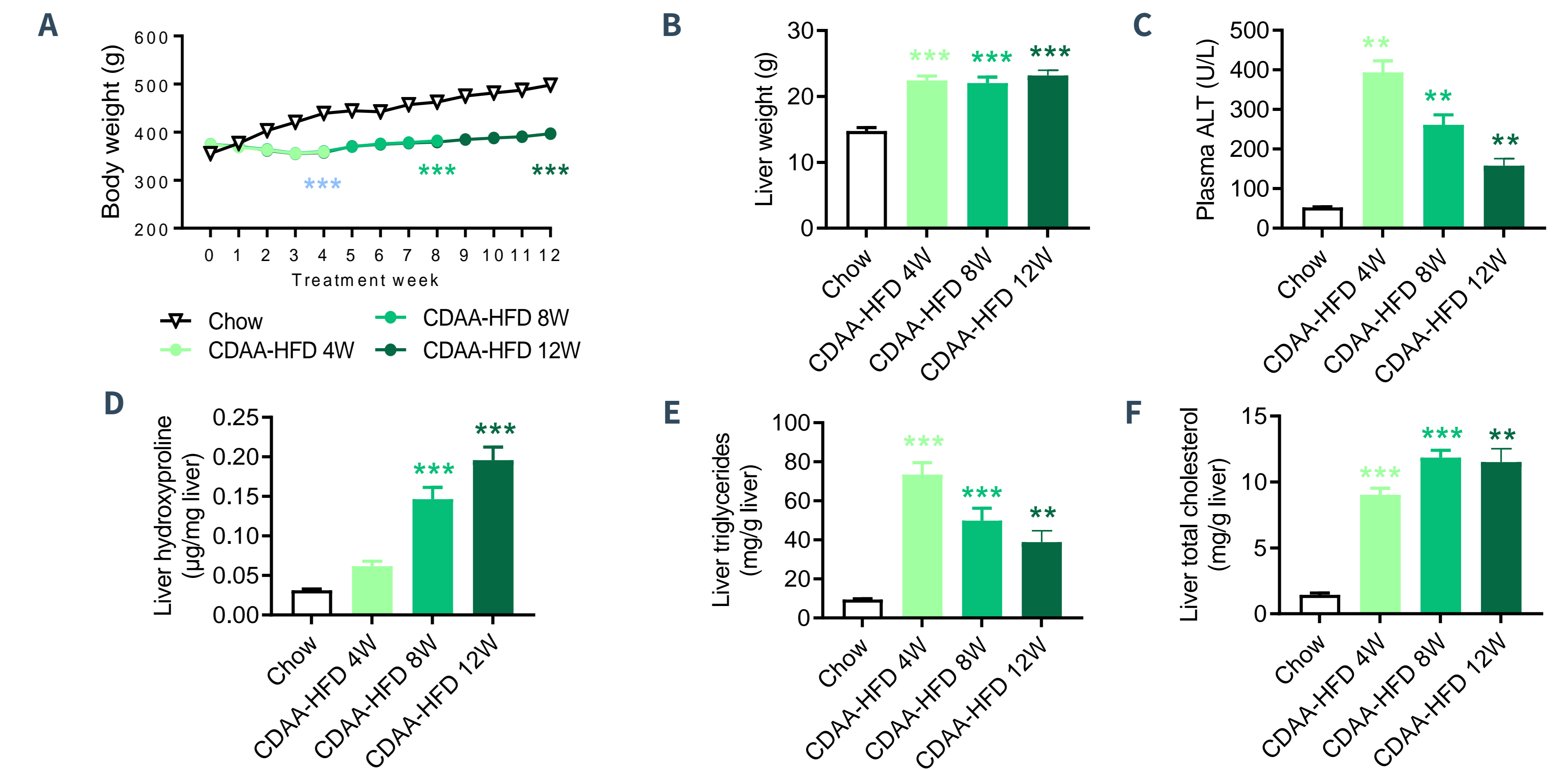## 2 Metabolic and biochemical parameters

Figure 2. CDAA-HFD induction reduces body weight, increases liver weight and worsen biochemical parameters.
(A) Body weight change during time. (B) Terminal liver weight. (C) Terminal plasma alanine aminotransferase (ALT). (D) Terminal liver hydroxyproline. (E) Terminal liver triglycerides. (F) Terminal liver total cholesterol. **$p<0.01$, ***$p<0.001$ compared to corresponding Chow control (Dunnett's test one-factor linear model).

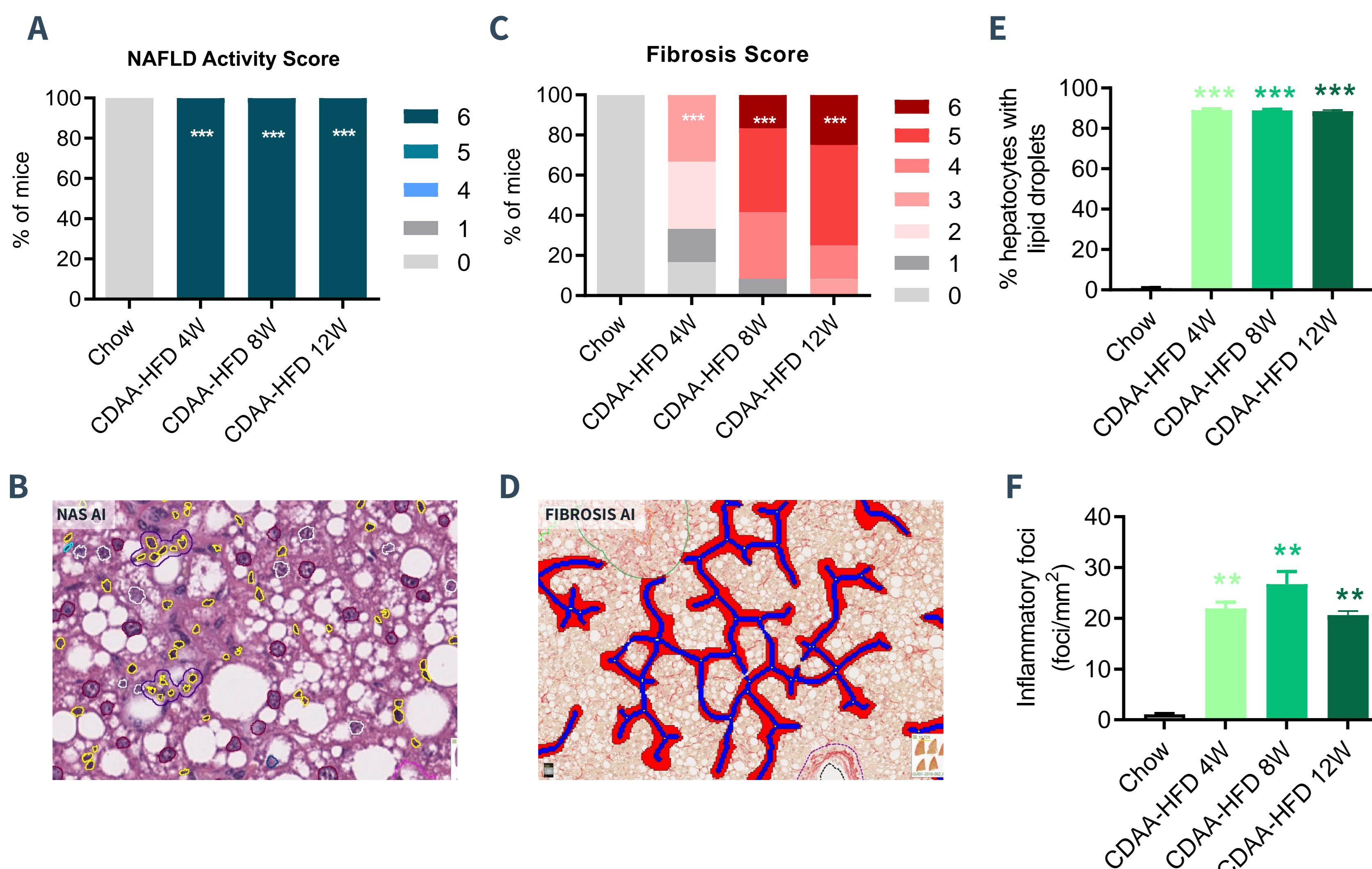## 3 Histopathological NAFLD Activity Score and Fibrosis Score

Figure 3. CDAA-HFD induction progressively worsen liver histopathological scores.
Histopathological scores and histomorphometry were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. (A) NAFLD Activity Score (NAS). (B) Representative HE photomicrographs used for GHOST evaluation. (C) Fibrosis Score (Ishak). (D) Representative PSR photomicrographs used for GHOST evaluation. (E) % hepatocytes with lipid droplets. (F) Number of inflammatory foci. ***$p<0.001$ compared to chow group (One-sided Fisher's exact test with Bonferroni correction).

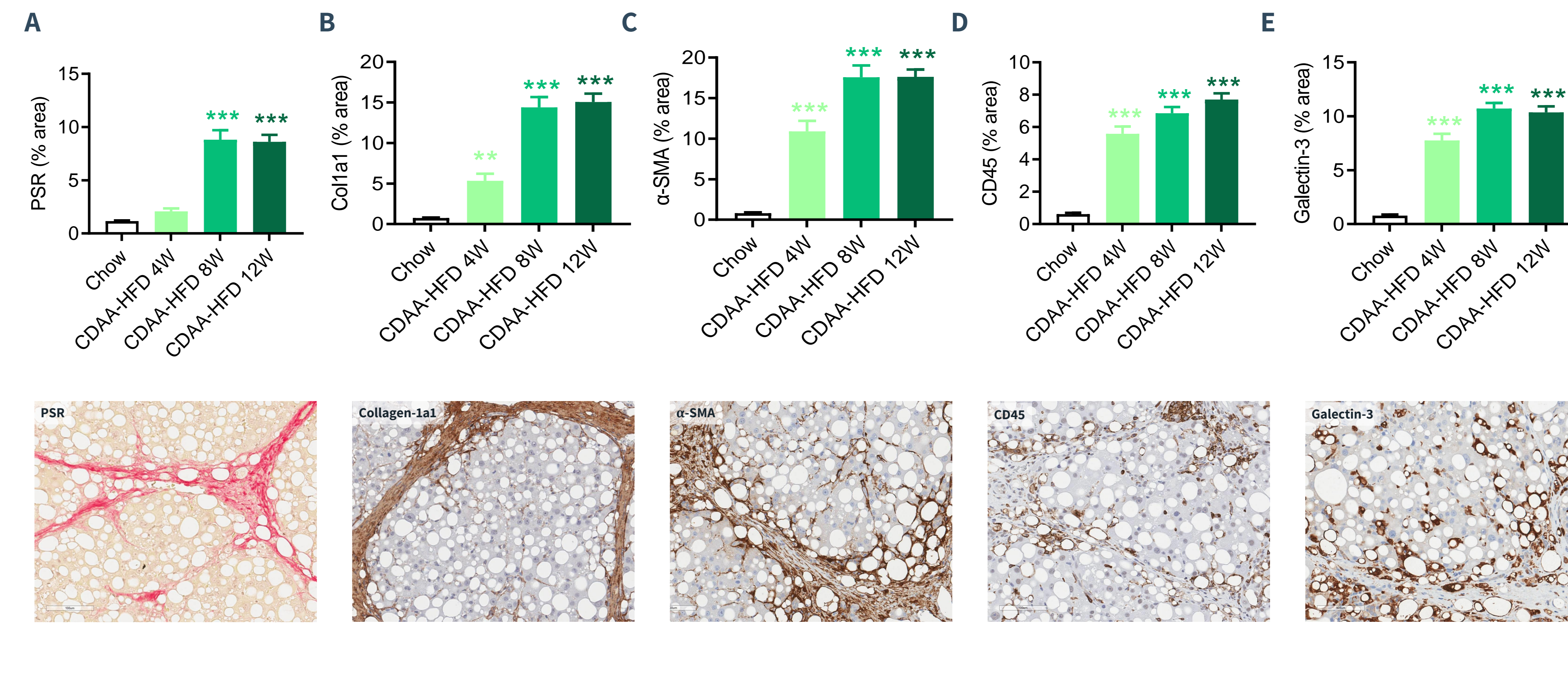## 4 Histological quantitative markers of fibrosis and inflammation

Figure 4. CDAA-HFD induction progressively worsen quantitative liver histological markers for fibrosis and inflammation.
Conventional IHC image analysis of: (A) % area of PSR; (B) % area of collagen-1a1; (C) % area of alpha-smooth muscle actin (α-SMA); (D) % area of CD45; (E) % area of galectin-3. Mean ± SEM. **$p<0.01$, ***$p<0.001$ compared to chow group (Dunnett's test one-factor linear model). Bottom panels: Representative PSR, collagen 1a1, α-SMA, CD45 and galectin-3, photomicrographs of CDAA-HFD 12 weeks group (scale bar, 100 µm).

CONCLUSION

+ CDAA-HFD induction reduces body weight, induces hepatomegaly and increases plasma ALT and liver TC and TG levels after 4 weeks of dieting.

+ CDAA-HFD induction induces liver steatosis and inflammation with advanced NAFLD Activity Score after 4 weeks of dieting.

+ CDAA-HFD induction progressively induces worsening in fibrosis with advanced fibrosis score and cirrhosis after 8-12 weeks of dieting.

+ CDAA-HFD induction progressively increases quantitative histological markers of inflammation, fibrosis and stellate cell activation.

+ CDAA-HFD non-obese rat model of advanced NASH allows for exploration of drug efficacy for progressive fibrosis.

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