Characterization of dietary intervention in the non-obese CDAA-HFD mouse model of advanced NASH with progressive fibrosis

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BACKGROUND & AIM

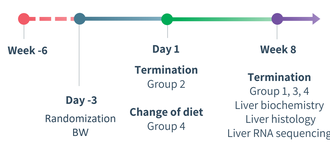
Current standard of care for non-alcoholic steatohepatitis (NASH) involves lifestyle modification, notably dietary intervention, aiming to promote regression or resolution of NASH and liver fibrosis.

We have recently characterized dietary intervention (chow reversal) in the translational GAN diet-induced obese (DIO) mouse model of fibrosing NASH (Møllerhøj et al. Clin Transl Sci, 2022). The present study aimed to evaluate chow-reversal in the non-obese choline-deficient L-amino-acid defined high-fat diet (CDAA-HFD) mouse model of advanced NASH with progressive fibrosis.

METHODS

C57BL/6JRj mice were fed chow or choline-deficient high-fat diet (CDAA-HFD, 45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 6 weeks before treatment start (i.e. after induction of fibrosis). Prior to treatment, animals were randomized into treatment groups based on body weight. A baseline group (n=12) was terminated at study start. Dietary intervention was performed by switching from CDAA-HFD to chow feeding on treatment day 1 (n=12). 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	Baseline CDAA-HFD	Baseline CDAA-HFD	12
3	Vehicle CDAA-HFD	Vehicle CDAA-HFD	12
4	Chow reversal	Chow reversal	12

2 Metabolic and biochemical parameters

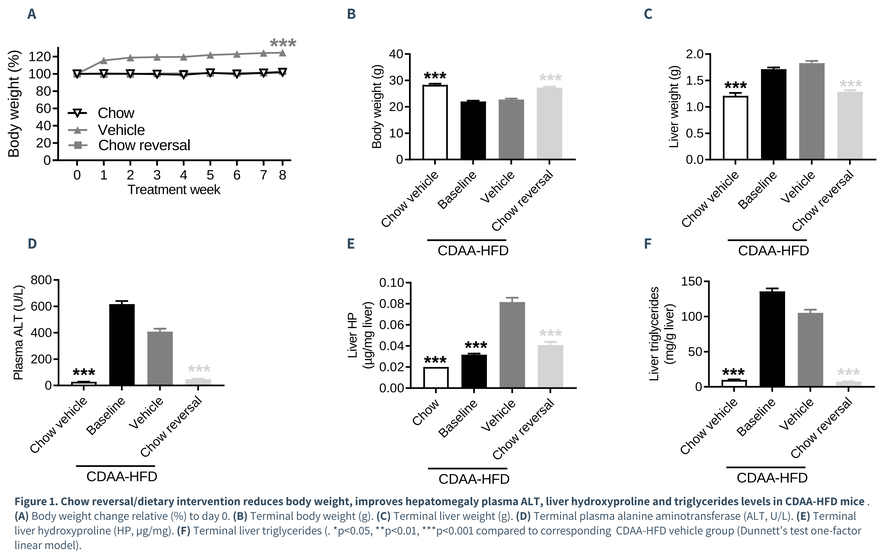


Figure 1. Chow reversal/dietary intervention reduces body weight, improves hepatomegaly plasma ALT, liver hydroxyproline and triglycerides levels in CDAA-HFD mice . **(A)** Body weight change relative (%) to day 0. **(B)** Terminal body weight (g). **(C)** Terminal liver weight (g). **(D)** Terminal plasma alanine aminotransferase (ALT, U/L). **(E)** Terminal liver hydroxyproline (HP, µg/mg). **(F)** Terminal liver triglycerides (. *p<0.05, **p<0.01, ***p<0.001 compared to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

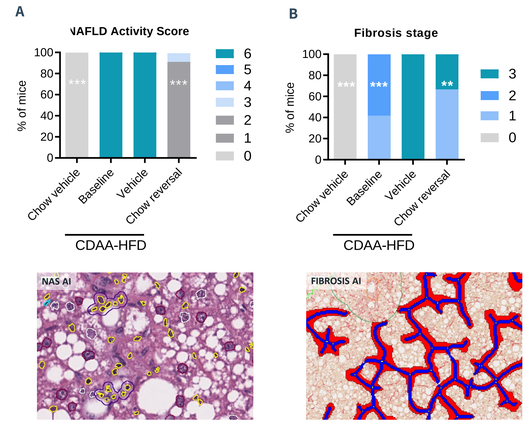


Figure 2. Chow reversal/dietary intervention improves NAFLD activity score and Fibrosis Stage in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. **p<0.01, ***p<0.001 compared to CDAA-HFD vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Quantitative histological markers of steatosis, inflammation and fibrogenesis

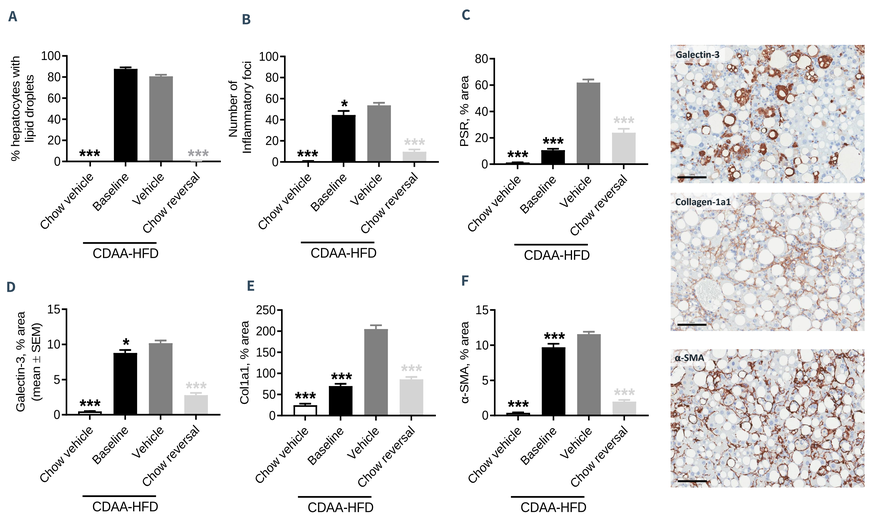


Figure 3. Chow reversal/dietary intervention improves quantitative histological markers of steatosis, inflammation and fibrosis in CDAA-HFD mice. . Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis (**A**) % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. *p<0.05, ~~**p<0.01,~~ ***p<0.001 compared to CDAA-HFD vehicle group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

5 Hepatic transcriptomic profile

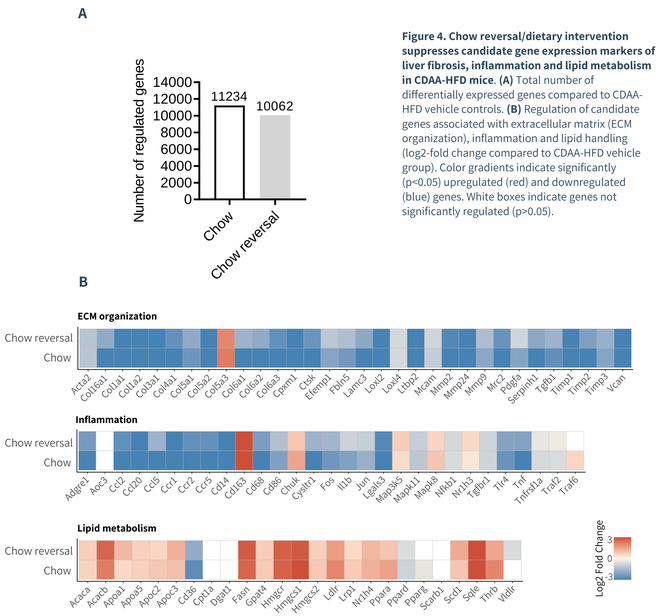


Figure 4. Chow reversal/dietary intervention suppresses candidate gene expression markers of liver fibrosis, inflammation and lipid metabolism in CDAA-HFD mice. **(A)** Total number of differentially expressed genes compared to CDAA-HFD vehicle controls. **(B)** Regulation of candidate genes associated with extracellular matrix (ECM organization), inflammation and lipid handling (log2-fold change compared to CDAA-HFD vehicle group). Color gradients indicate significantly (p<0.05) upregulated (red) and downregulated (blue) genes. White boxes indicate genes not significantly regulated (p>0.05).

CONCLUSION

Chow reversal/dietary intervention in CDAA-HFD mice:

+ Normalizes body and liver weight as well as plasma, liver biochemistry and liver hydroxyproline levels.
+ Improves both NAFLD Activity Score and fibrosis stage.
+ Reduces quantitative histological markers of steatosis, inflammation and fibrosis
+ Suppresses hepatic genes linked to inflammation and fibrosis

Effects of dietary intervention in the non-obese CDAA-HFD mouse model of NASH with progressive fibrosis are in good agreement with clinical outcomes in NASH patients.