# Characterization of semaglutide treatment in the non-obese CDAA-HFD mouse model of advanced NASH with progressive fibrosis


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## BACKGROUND & AIM

The long-acting glucagon-like peptide-1 (GLP-1) analogue semaglutide is approved for the treatment of type 2 diabetes and obesity. Recently, semaglutide has been reported to improve liver histological outcomes in patients with non-alcoholic steatohepatitis (NASH) and fibrosis (Newsome *et al.*, NEJM, 2020). Semaglutide is currently in phase-3 clinical trial (ESSENCE) for the treatment of NASH.

We have recently characterized semaglutide treatment 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 semaglutide treatment 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. CDAA-HFD fed mice (n=12 per group) received treatment (SC, QD) with vehicle or semaglutide (30 nmol/kg) for 8 weeks. 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 | 12 |
| 3 | Vehicle CDAA-HFD | Vehicle | 12 |
| 4 | Semaglutide CDAA-HFD | Semaglutide | 12 |

## 2 Metabolic and biochemical parameters

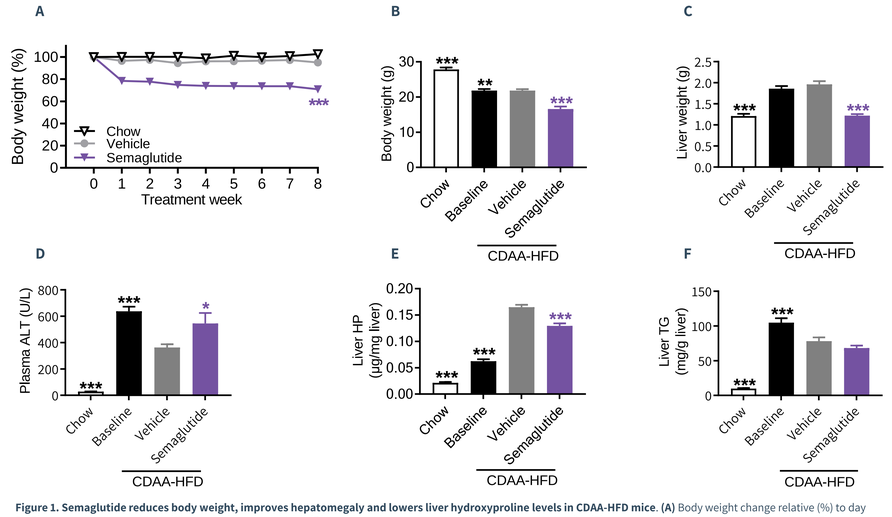


**Figure 1. Semaglutide reduces body weight, improves hepatomegaly and lowers liver hydroxyproline levels in CDAA-HFD mice**. **(A)** Body weight change relative (%) to day 0. **(B)** Terminal body weight (g). **(C)** Terminal liver weight (g). **(D)** Terminal plasma alanine aminotransferase (ALT, U/L).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

## 3 NAFLD Activity Score and Fibrosis Stage

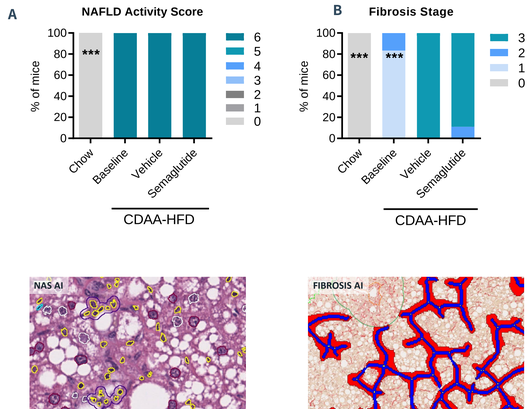





**Figure 2. Semaglutide does not influence histopathological scores in CDAA-HFD mice**. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. ***p<0.001 compared 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

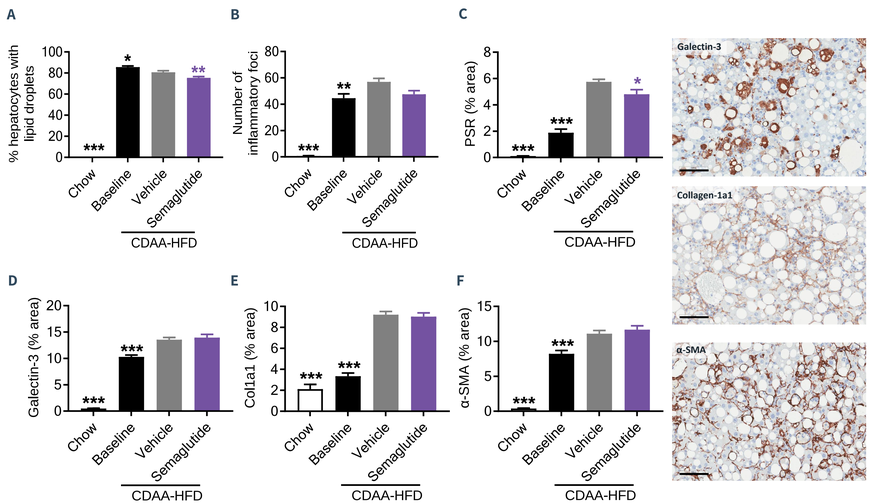


**Figure 3. Semaglutide marginally improves quantitative histological markers of steatosis and fibrosis in CDAA-HFD mice**. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis (**A**) % hepatocytes 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 Liver transcriptome profile

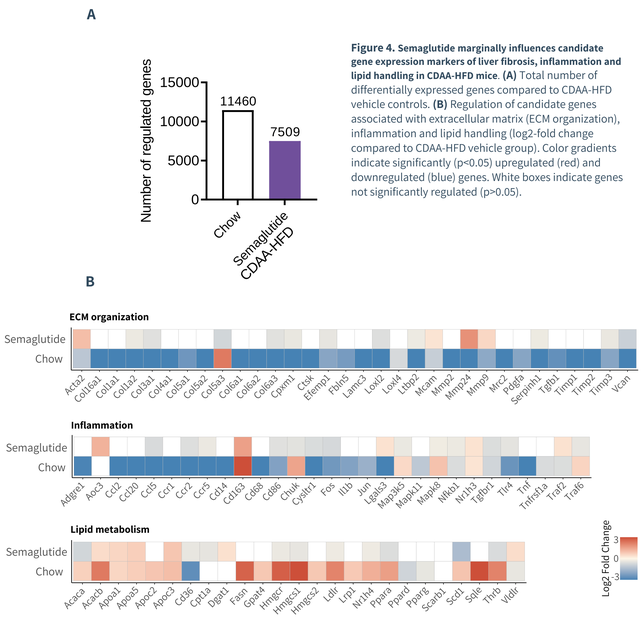


**Figure 4. Semaglutide marginally influences candidate gene expression markers of liver fibrosis, inflammation and lipid handling in CDAA-HFD mice**. **(A)** Total number of differentially expressed genes compared to CDAA-HFD vehicle controls. **(B)** Regulation of candidate genes associated with extracellular 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

Semaglutide treatment in CDAA-HFD mice:

+ Reduces body weight and hepatomegaly
+ Slightly reduces liver hydroxyproline levels
+ Shows no effect on NAFLD Activity Score and fibrosis stage
+ Marginally improves quantitative histological markers of steatosis and fibrosis
+ Minimally influences hepatic genes linked to fibrosis and inflammation

Semaglutide demonstrates very limited therapeutic efficacy in the non-obese CDAA-HFD mouse model of NASH with progressive fibrosis, hence contrasting clinical trial outcomes in NASH patients.