Prophylactic and therapeutic hepatoprotective effects of semaglutide in the 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. Semaglutide has recently 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.

The present study aimed to evaluate prophylactic vs. therapeutic intervention with semaglutide 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 CDAA-HFD (45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 3 or 6 weeks prior to treatment start (i.e. before or after onset of fibrosis, respectively). Animals were randomized into treatment groups based on body weight. A baseline group (n=12) was terminated at study start (3 and 6 weeks). CDAA-HFD fed mice (n=9 - 12 per group) received treatment (SC) with vehicle or semaglutide (30 nmol/kg) for 9 weeks (prophylactic, 12w on diet) or 8 weeks (therapeutic, 14w on diet). Chow-fed mice (n=8) served as normal controls. Terminal endpoints included plasma biomarkers [alanine/aspartate aminotransferase (ALT/AST)], liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage, quantitative liver histology and liver RNA sequencing.

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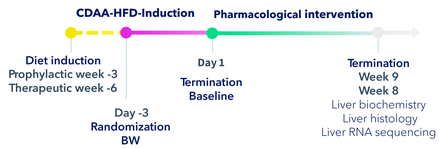
1 Study Outline



Group no.	Group	Name	Number of animals	Administration route	Dosing frequency	Dosing concentration
1	Chow	**Chow**	8	SC	QD	-
2	Baseline CDAA-HFD 3w	**Baseline 3w**	12	-	-	-
3	Vehicle CDAA-HFD 12w	**Vehicle 12w**	12	SC	QD	-
4	Semaglutide CDAA-HFD 12w	**Semaglutide 12w**	11	SC	QD	30 nmol/kg
5	Baseline CDAA-HFD 6w	**Baseline 6w**	12	-	-	-
6	Vehicle CDAA-HFD 14w	**Vehicle 14w**	12	SC	QD	-
7	Semaglutide CDAA-HFD 14w	**Semaglutide 14w**	9	SC	QD	30 nmol/kg

2 Metabolic and biochemical parameters

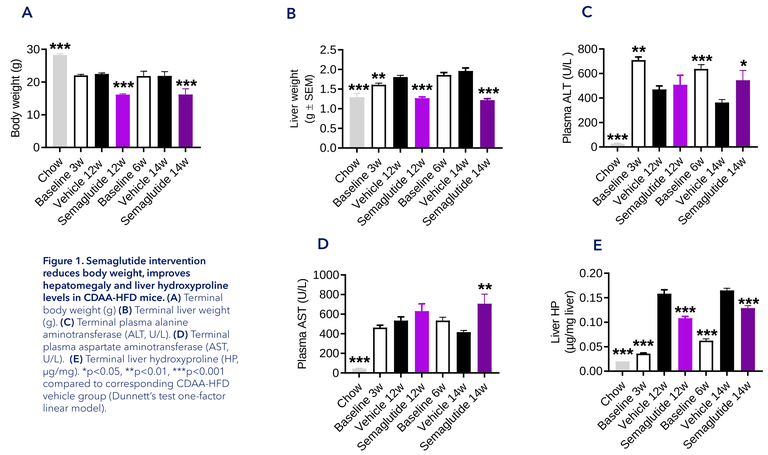


Figure 1. Semaglutide intervention reduces body weight, improves hepatomegaly and liver hydroxyproline levels in CDAA-HFD mice. (A) Terminal body weight (g) **(B)** Terminal liver weight (g). **(C)** Terminal plasma alanine aminotransferase (ALT, U/L). **(D)** Terminal plasma aspartate aminotransferase (AST, U/L). **(E)** Terminal liver hydroxyproline (HP, µg/mg). *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

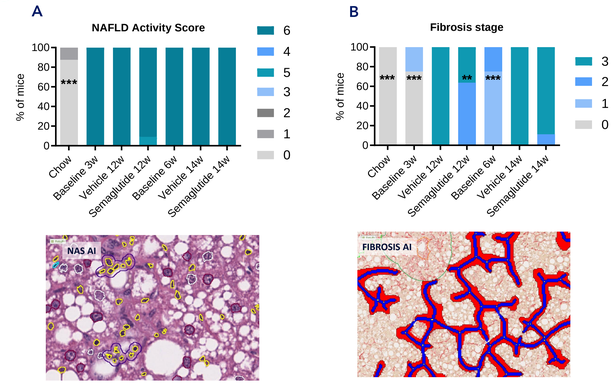


Figure 2. Prophylactic semaglutide intervention improves fibrosis stage (early-stage), in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. **p<0.01, ***p<0.001 compared to corresponding 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

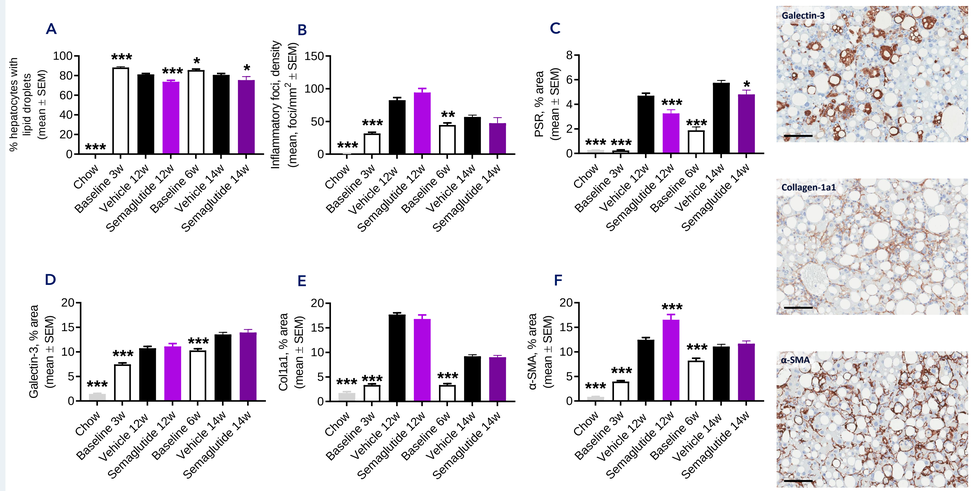


Figure 3. Semaglutide intervention improves quantitative histological markers of steatosis and fibrosis in CDAA-HFD mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis **(A)** % hepatocytes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

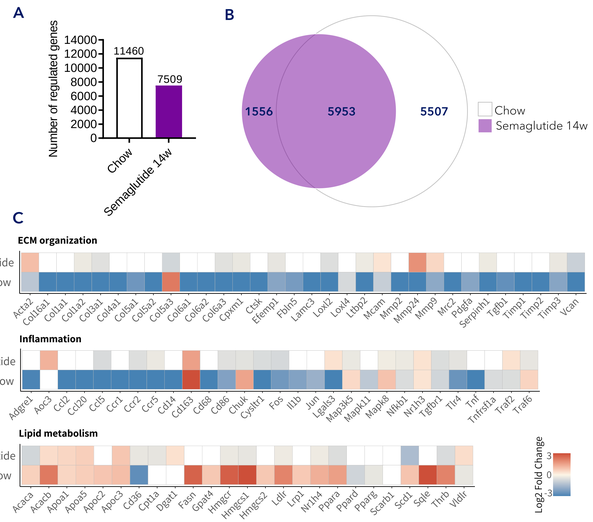


Figure 4. Semaglutide marginally influences candidate gene expression markers of liver fibrosis, inflammation and lipid handling in CDAA-HFD mice. (A) Total number of differentially expressed genes compared to CDAA-HFD vehicle controls. **(B)** Visualization of the number of genes regulated by combinations of compounds compared to CDAA-HFD **(C)** 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 outcomes in CDAA-HFD mice:

+ Prophylactic and therapeutic intervention reduces body weight and improves hepatomegaly and liver hydroxyproline levels
+ No effect was seen on NAFLD Activity Score
+ Prophylactic and therapeutic intervention improves quantitative histological markers of steatosis and fibrosis
+ Only prophylactic intervention improves Fibrosis Stage
+ Therapeutic intervention marginally influences hepatic genes linked to lipid, inflammation and ECM organization

Semaglutide intervention 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.