Proof-of-concept testing of semaglutide in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

Authors
Jacob Nøhr-Meldgaard, Ditte D. Thorbeck, Denise Oro, Martin Rønn Madsen, Henrik H. Hansen, Michael Feigh.

Gubra, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

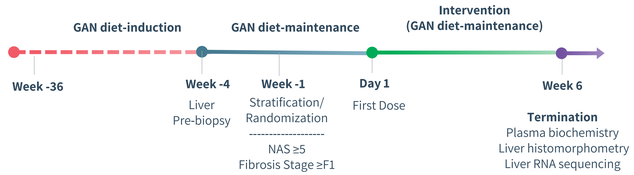
BACKGROUND & AIM

The long-acting glucagon-like peptide-1 (GLP-1) analogue semaglutide is approved for the treatment of type 2 diabetes and obesity. In a recent clinical phase 3 tirl (ESSENCE), semaglutide has been reported to improve liver histopathological outcomes in patients with metabolic dysfunction-associated steatohepatitis (MASH) and fibrosis (Sanyal et al., NEJM; 2025). We have recently showed therapeutic efficacy during longer-term semaglutide intervention in the translational GAN diet-induced obese (DIO) mouse model of fibrosing MASH (Møllerhøj et al. Clin Transl Sci, 2022).
The present study aimed to evaluate semaglutide treatment for 6 weeks proof-of-concept testing in the biopsy-confirmed GAN DIO-MASH mouse model.

METHODS

C57BL/6JRj mice were fed the GAN diet for 36 weeks before treatment start. All animals underwent liver pre-biopsy collection 4-weeks prior to treatment start. Only histology-confirmed animals with NAFLD Activity Score (NAS) ≥5 and Fibrosis Stage ≥F1 were included. GAN DIO-MASH fed mice received treatment with vehicle or semaglutide for 6 weeks. Terminal endpoints included plasma biochemistry, quantitative liver histology and RNAseq with bioinformatic analysis.

1 Study outline



Group no.	Group	Name	Number of animals	Treatment	Administration route	Dosing Frequency	Dose
1	GAN DIO-MASH	Vehicle	12	Vehicle	SC	QD	-
2	GAN DIO-MASH	Semaglutide	12	Semaglutide	SC	QD	30nmol/kg

2 Metabolic and biochemical parameters

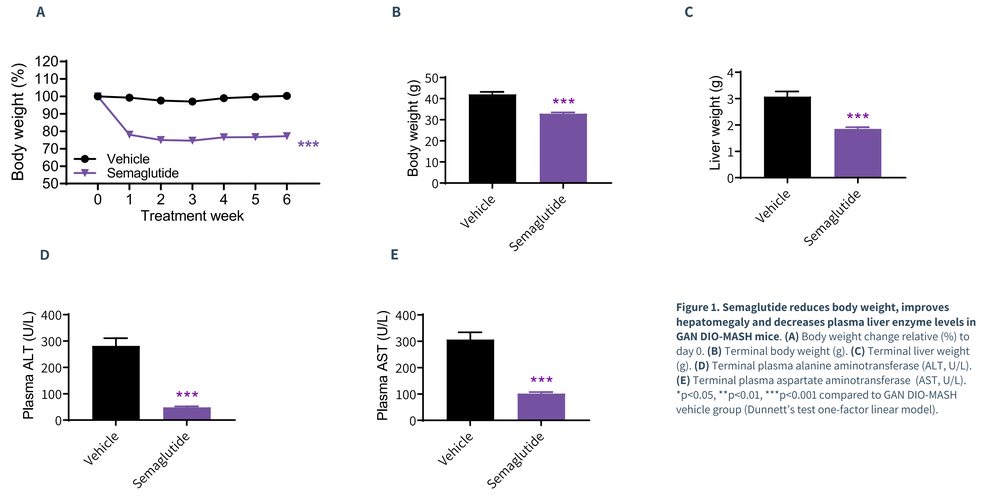


Figure 1. Semaglutide reduces body weight, improves hepatomegaly and decreases plasma liver enzyme levels in GAN DIO-MASH mice. **(A)** Body weight change relative (%) to day 0. **(B)** Terminal body weight (g). **(C)** Terminal liver weight (g). **(D)** Terminal plasma alanine aminotransferase (ALT, U/L). **(E)** Terminal plasma aspartate aminotransferase (AST, U/L). *p<0.05, **p<0.01, ***p<0.001 compared to GAN DIO-MASH vehicle group (Dunnett's test one-factor linear model).

3 Quantitative histological markers of steatosis, inflammation and fibrogenesis

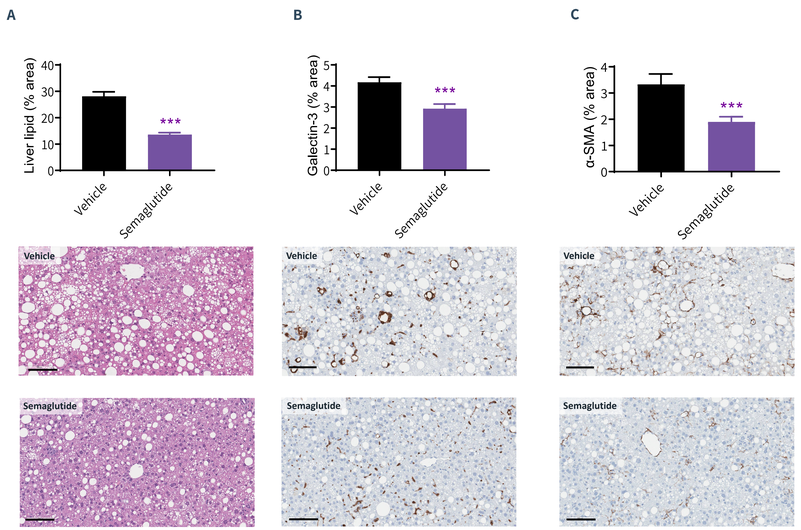


Figure 2. Semaglutide improves quantitative histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-MASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis **(A)** % area of liver lipids. **(B) %** area of galectin-3 **(C)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared to GAN DIO-MASH vehicle group (Dunnett's test one-factor linear model). Right panels: Representative Liver lipid, galectin-3 and α-SMA photomicrographs (scale bar, 100 µm).

4 Hepatic transcriptomic profile

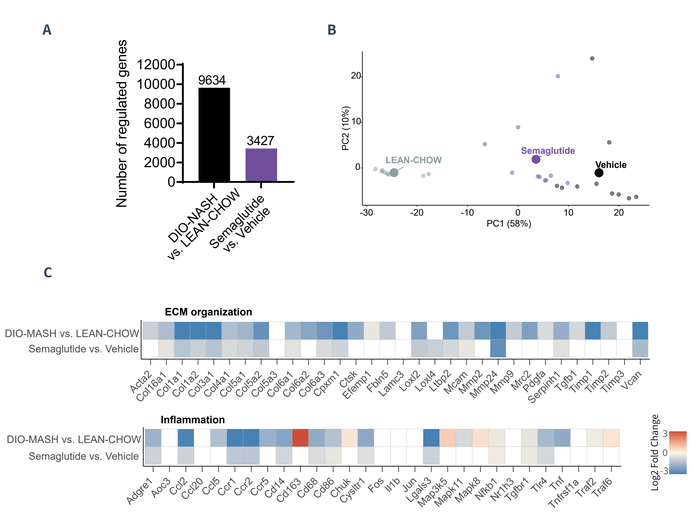


Figure 3. Semaglutide influences candidate gene expression markers of liver fibrosis and inflammation in GAN DIO-MASH mice. **(A)** Total number of differentially expressed genes compared to historical LEAN-CHOW control and DIO-MASH vehicle. **(B)** Principal component analysis (PCA) of the 500 most variable genes. **(C)** Regulation of candidate genes associated with extracellular matrix (ECM organization), inflammation and lipid handling (log2-fold change compared to DIO-MASH 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 therapeutic intervention for 6-weeks demonstrated:

+ reduced body weight, hepatomegaly, plasma ALT and AST levels.
+ reduced quantitative histological markers of steatosis, inflammation and fibrogenesis.
+ Induced profound impact on hepatic transcriptomic profile and regulated genes involved in ECM and inflammation.
+ The GAN DIO-MASH mouse model allows for proof-of-concept testing for shorter duration of treatment.