Metabolic, biochemical, histological, and transcriptomic effects of semaglutide in the GAN diet-induced and biopsy-confirmed ob/ob mouse model of NASH

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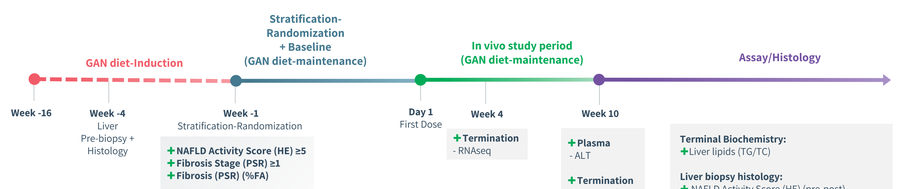
BACKGROUND & AIM

The glucagon-like-peptide (GLP)-1 analogue semaglutide, currently approved for the treatment of type 2 diabetes and obesity, is in advanced clinical development for non-alcoholic steatohepatitis (NASH). In line with clinical findings, semaglutide has been demonstrated to improve parameters of NASH including NAFLD Activity Score in the GAN DIO-NASH mouse model.

The present study aimed to evaluate the metabolic, biochemical, histological and transcriptomic effects of 10 weeks treatment with semaglutide in the GAN ob/ob-NASH mouse model with progressive fibrosis.

1 Study outline



Group #	Group name	Number of Animals	Animal model	Dose	Dosing frequency	Route of administration
1	Vehicle	17	ob/ob-NASH	NA	QD	SC
2	Semaglutide	17	ob/ob-NASH	30 nmol/kg	QD	SC

Figure 1. Study outline, groups and treatments. ALT: alanine aminotransferase; TC: total cholesterol; TG: triglycerides; QD: once daily; SC: subcutaneous; PO: per oral.

2 Metabolic and biochemical parameters

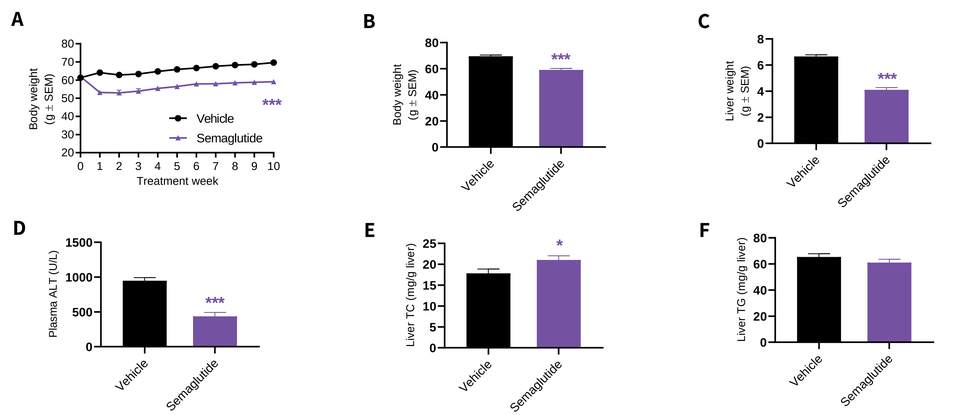


Figure 2. Semaglutide improves metabolic and biochemical parameters in GAN ob/ob-NASH mice. **(A)** Absolute body weight during study period. **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(D)** Terminal plasma aspartate aminotransferase (AST). **(E)** Terminal liver total cholesterol. **(F)** Terminal liver triglycerides. *p<0.05, ***p<0.001 compared to corresponding vehicle control (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis stage

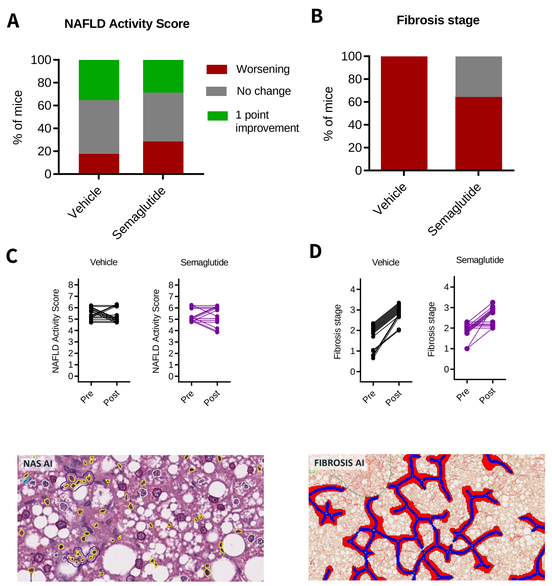


Figure 3. Semaglutide has no effect on histopathological scores in GAN ob/ob-NASH mice.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C, D)** Comparison of individual pre-post NAS and individual pre-post Fibrosis stage. Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Histological quantitative markers of steatosis, inflammation and fibrosis

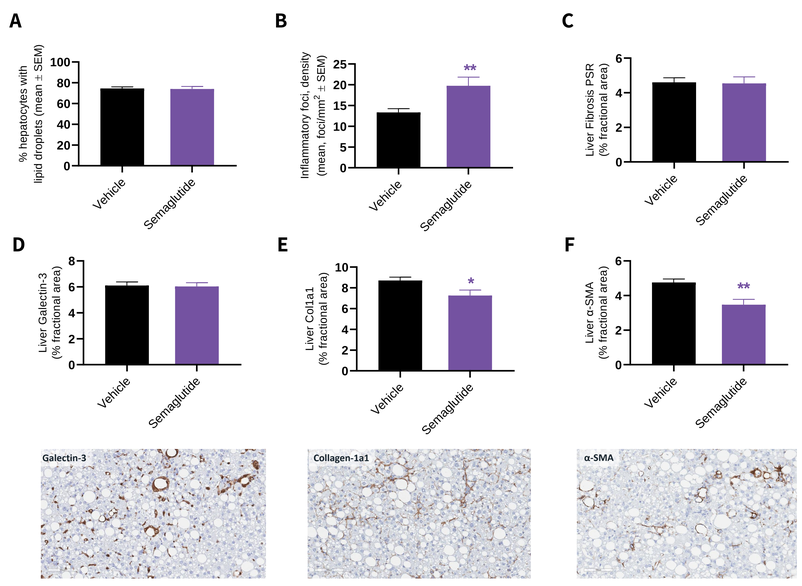


Figure 4. Semaglutide decreases histological markers for fibrogenesis in GAN ob/ob-NASH mice.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). (**A**) % hepatocytes with 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) as marker for stellate cell activation. Mean ± SEM. *p<0.05, *p<0.05 **p<0.01 to corresponding vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for semaglutide treatment group (scale bar, 100 µm).

5 Transcriptomic profile for fibrosis and inflammation

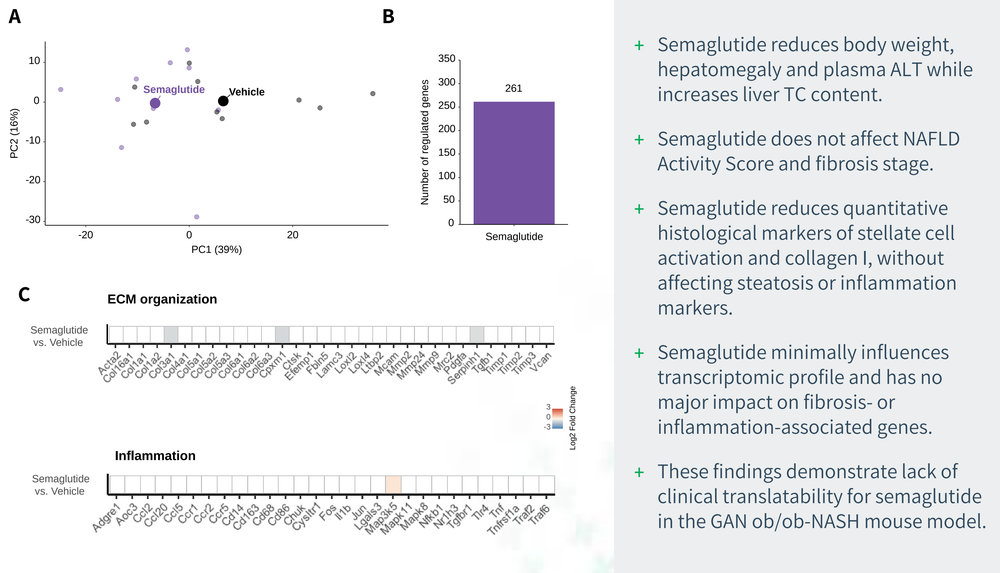


Figure 5. Semaglutide does not affect fibrosis- or inflammation-associated genes in GAN ob/ob-NASH mice.
(A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Differentially expressed genes. **(C)** Regulation of hepatic extracellular matrix (ECM) and inflammation candidate genes (log2-fold change compared to corresponding DIO-NASH vehicle control mice). Blue and red colour gradients indicate significantly (p<0.05) down-regulated and up-regulated gene expression, respectively. White boxes indicate genes not significantly regulated (p>0.05).

CONCLUSION

+ Semaglutide reduces body weight, hepatomegaly and plasma ALT while increases liver TC content.
+ Semaglutide does not affect NAFLD Activity Score and fibrosis stage.
+ Semaglutide reduces quantitative histological markers of stellate cell activation and collagen I, without affecting steatosis or inflammation markers.
+ Semaglutide minimally influences transcriptomic profile and has no major impact on fibrosis- or inflammation-associated genes.
+ These findings demonstrate lack of clinical translatability for semaglutide in the GAN ob/ob-NASH mouse model.

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