Hepatoprotective effects of semaglutide in the diet-induced obese Ldlr$^{-/-}$ mouse model of NASH

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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 DIO low-density lipoprotein (LDL) receptor knock-out (DIO-LDLR-KO) mouse model of NASH.

METHODS

Male LDLR$^{-/-}$ mice fed western diet (D12079B Research diets, 41 %-kcal fat, 0.21% cholesterol) for 17 weeks and administered (SC, QD) vehicle or semaglutide (30 nmol/kg) for 17 weeks as prophylactic intervention. Chow-fed LDLR$^{-/-}$ mice served as controls. Terminal endpoints included body weight, plasma and liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage and quantitative liver histology.

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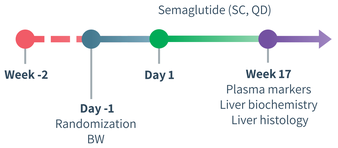
1 Study outline



Group	Animal model	Treatment	Number of animals
1	CHOW-LDLR-KO	Vehicle	12
2	DIO-LDLR-KO	Vehicle	16
3	DIO-LDLR-KO	Semaglutide (30 nmol/kg)	15

2 Metabolic and biochemical parameters

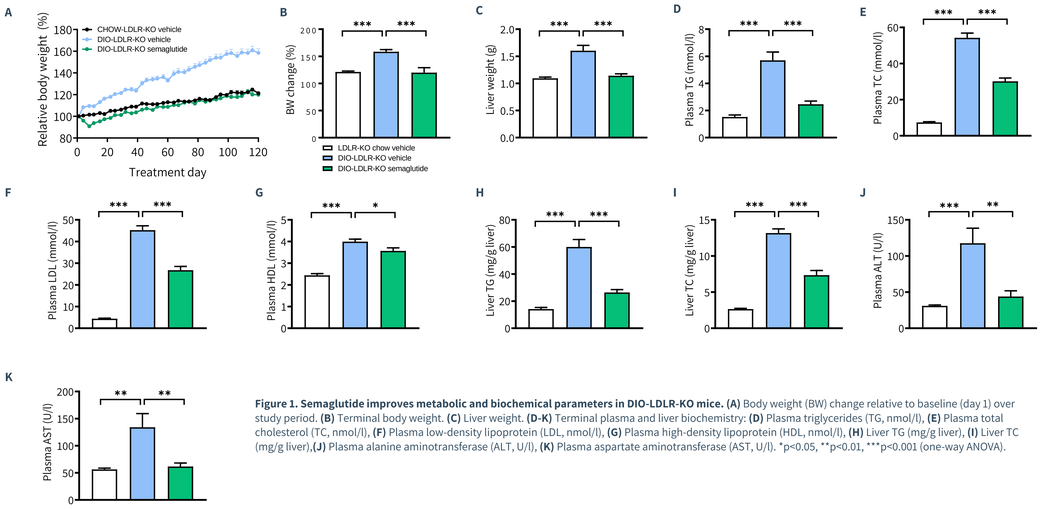


Figure 1. Semaglutide improves metabolic and biochemical parameters in DIO-LDLR-KO mice. (A) Body weight (BW) change relative to baseline (day 1) over study period. **(B)** Terminal body weight. **(C)** Liver weight. **(D-K)** Terminal plasma and liver biochemistry: **(D)** Plasma triglycerides (TG, nmol/l), **(E)** Plasma total cholesterol (TC, nmol/l), **(F)** Plasma low-density lipoprotein (LDL, nmol/l), **(G)** Plasma high-density lipoprotein (HDL, nmol/l), **(H)** Liver TG (mg/g liver), **(I)** Liver TC (mg/g liver),**(J)** Plasma alanine aminotransferase (ALT, U/l), **(K)** Plasma aspartate aminotransferase (AST, U/l). *p<0.05, **p<0.01, ***p<0.001 (one-way ANOVA).

3 NAFLD Activity Score and Fibrosis Stage

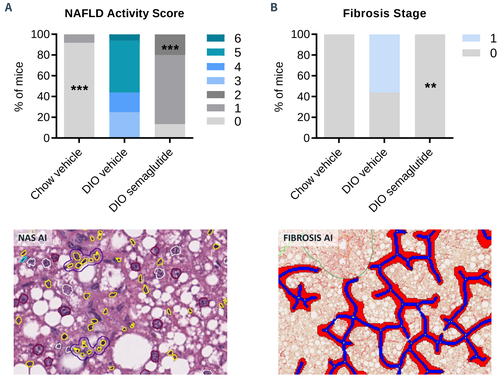


Figure 2. Semaglutide improves liver histopathological scores in DIO-LDLR-KO mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. **p<0.01, ***p<0.001 compared to DIO-LDLR-KO vehicle group (one-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Steatosis, inflammation and ballooning degeneration scores

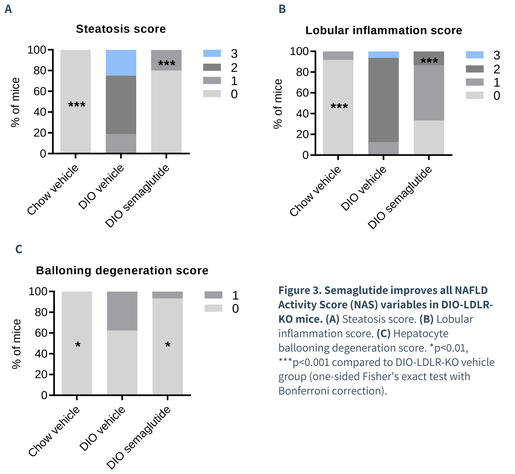


Figure 3. Semaglutide improves all NAFLD Activity Score (NAS) variables in DIO-LDLR-KO mice. (A) Steatosis score. **(B)** Lobular inflammation score. **(C)** Hepatocyte ballooning degeneration score. *p<0.01, ***p<0.001 compared to DIO-LDLR-KO vehicle group (one-sided Fisher's exact test with Bonferroni correction).

5 Quantitative histological markers of steatosis, inflammation and fibrosis

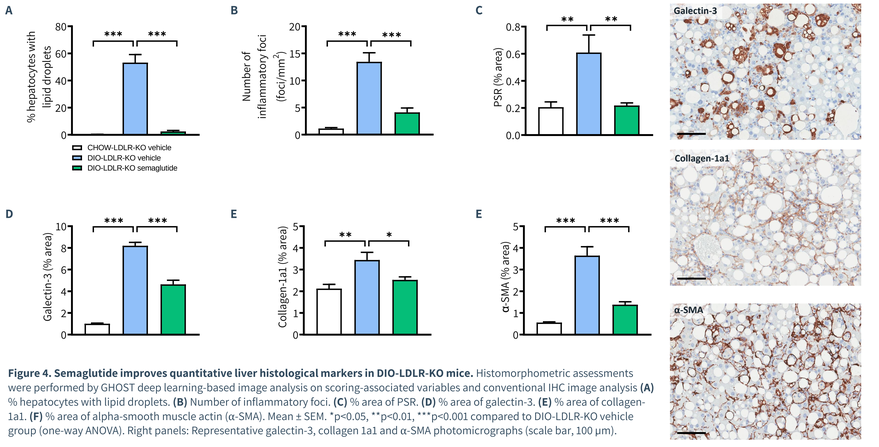


Figure 4. Semaglutide improves quantitative liver histological markers in DIO-LDLR-KO mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis **(A)** % hepatocytes with lipid droplets. **(B)** Number 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 DIO-LDLR-KO vehicle group (one-way ANOVA). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

CONCLUSION

- Semaglutide induces weight loss, improves hepatomegaly, dyslipidemia, and transaminases in DIO-LDLR-KO mice
- Semaglutide improves both NAS and fibrosis scores in DIO-LDLR-KO mice
- Semaglutide improves quantitative histological markers of steatosis, inflammation and fibrosis in DIO-LDLR-KO mice

The DIO-LDLR-KO mouse represents a translational model for evaluating drug effects on clinical and histological endpoints in NASH