Metabolic, biochemical and histopathological effects of long-term treatment with semaglutide in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

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Background & Aim

Semaglutide, a long-acting glucagon-like receptor 1 (GLP-1) agonist, approved for treatment of type 2 diabetes and obesity, is currently in late-stage clinical development for NASH. The present study aimed to evaluate the metabolic, biochemical and histopathological effects of long-term semaglutide treatment in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of fibrosing NASH.

Study outline

Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	LEAN-CHOW	Male	C57BL/6JRj	10	**Vehicle**	SC	QD	5 ml/kg	-
2	DIO-NASH	Male	C57BL/6JRj	17	**Vehicle**	SC	QD	5 ml/kg	-
3	DIO-NASH	Male	C57BL/6JRj	18	**Semaglutide**	SC	QD	5 ml/kg	30 nmol/kg

Improvement in metabolic and glycemic parameters

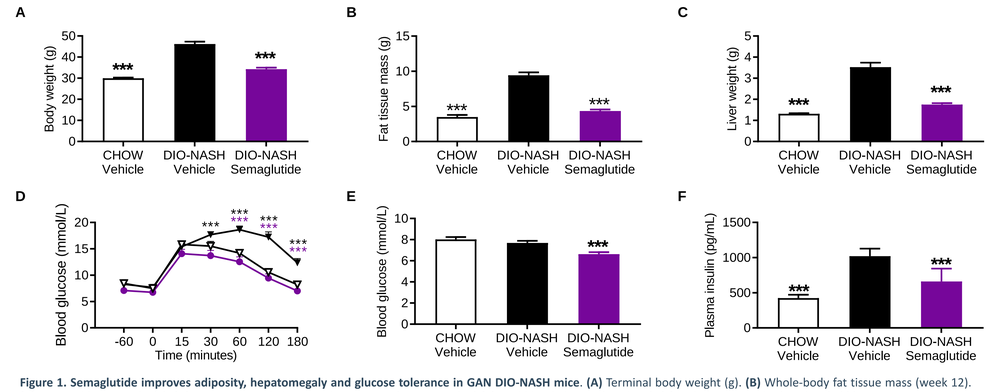

Figure 1. Semaglutide improves adiposity, hepatomegaly and glucose tolerance in GAN DIO-NASH mice. **(A)** Terminal body weight (g). **(B)** Whole-body fat tissue mass (week 12). **(B)** Terminal liver weight (g). **(D)** Intraperitoneal glucose tolerance test (ipGTT, week 8) **(E)**. Fed blood glucose (week 12). **(F)** Fed plasma insulin (week 12). ***p<0.001 compared to DIO-NASH vehicle group (Dunnett's test one-factor linear model).

Improvement in NAFLD Activity Score

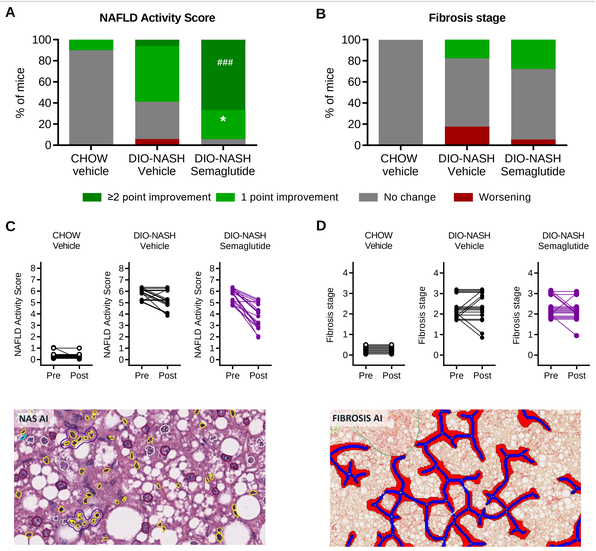

Figure 2. Semaglutide improves liver histopathological NAFLD Activity Score in GAN DIO-NASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Comparison of individual pre-post Fibrosis Stage. *p<0.05 with one-point improvement, ###p<0.001 with more than 2-point improvement compared to corresponding DIO-NASH vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

Improvement in quantitative histology of steatosis, inflammation and fibrosis

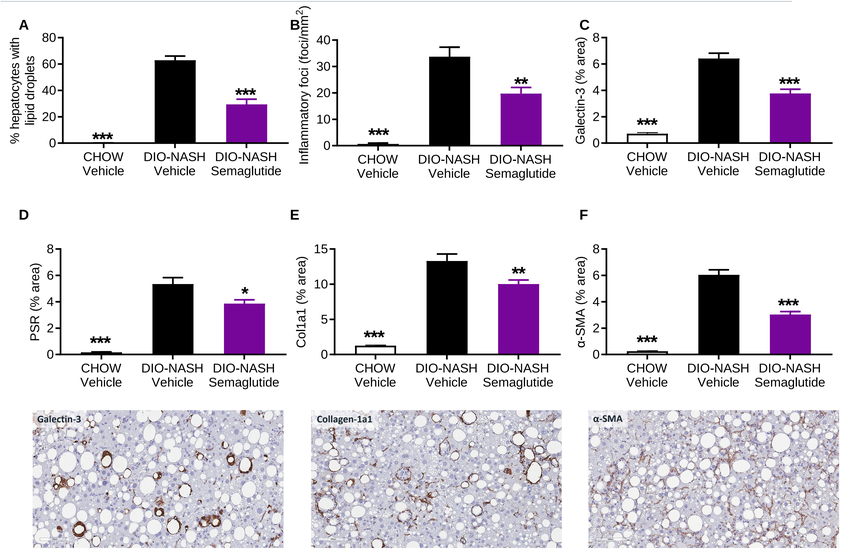

Figure 3. Semaglutide improves quantitative liver histological markers in GAN DIO-NASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3. **(D)** % area of PSR. **(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 to DIO-NASH vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

Improvement in plasma and liver biochemistry

	CHOW Vehicle	DIO-NASH Vehicle	DIO-NASH Semaglutide
Plasma ALT (U/L)	36.0 ± 1.8	309 ± 28	66.9 ± 7.6***
Plasma AST (U/L)	63.3 ± 4.4***	351 ± 32.1	122 ± 7.8***
Plasma ALP (U/L)	90.7 ± 2.4***	194 ± 10	99 ± 5.6***
Plasma TG (mmol/L)	0.7 ± 0.1***	0.5 ± 0.1	0.4 ± 0.1**
Plasma TC (mmol/L)	2.5 ± 0.1***	8.6 ± 0.4	4.9 ± 0.2***
Plasma HDL (mmol/L)	2.1 ± 0.1***	6.4 ± 0.2	3.7 ± 0.2***
Plasma LDL (mmol/L)	0.3 ± 0.1***	2.1 ± 0.3	1.1 ± 0.1***
Plasma TNF-α (pg/mL)	3.9 ± 0.5***	13.0 ± 1.3	6.8 ± 0.9***
Plasma MCP-1 (pg/mL)	5.6 ± 0.6***	17.9 ± 0.8	9.5 ± 1.5***
Plasma CK18 (ng/mL)	0.3 ± 0.1***	3.6 ± 0.4	1.1 ± 0.2***
Plasma TIMP-1 (ng/mL)	1.2 ± 0.1***	3.2 ± 0.2	1.7 ± 0.1***
Plasma PIINP (ng/mL)	0.6 ± 0.1***	3.7 ± 0.3	1.3 ± 0.1***
Liver TG (mg/g liver)	15.6 ± 0.9***	112 ± 7.6	55.7 ± 3.1***
Liver TC (mg/g liver)	2.5 ± 0.1***	11.0 ± 0.7	7.6 ± 0.4***

Table 1. Semaglutide improves plasma and liver biochemistry in GAN DIO-NASH mice. Abbreviations: ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; MCP-1, monocyte chemoattractant protein 1; TC, total cholesterol, TG, triglycerides; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF- α, tumor necrosis factor-α. Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 to DIO-NASH vehicle group (Dunnett's test one-factor linear model).

CONCLUSION

- Semaglutide reduces body weight, adiposity and hepatomegaly.
- Semaglutide improves glucose tolerance and hyperinsulinemia.
- Semaglutide improves plasma and liver biochemistry.
- Semaglutide promotes ≥2-point significant improvement in NAFLD Activity Score.
- Fibrosis stage was unaffected by semaglutide.
- Semaglutide reduces quantitative histological markers of steatosis, inflammation and fibrosis.
- These findings agree with clinical findings, further highlighting clinical translatability of the GAN DIO-NASH mouse model.