Metabolic, biochemical, histopathological, and transcriptomic effects of dietary intervention in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

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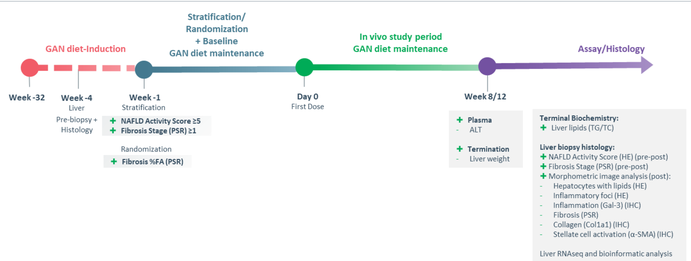
Background & Aim

Current standard of care for NASH involves lifestyle modification, notably dietary intervention, aiming to promote regression or resolution of non-alcoholic steatohepatitis (NASH) and liver fibrosis. The present study aimed to evaluate the metabolic, biochemical, histopathological and transcriptomic effects of dietary intervention (chow-reversal) in the Gubra-Amylin NASH (GAN) 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 concentrations
1	LEAN-CHOW	Male	C57BL/6JRj	6	Vehicle	SC	QD	5 ml/kg	NA
2	DIO-NASH	Male	C57BL/6JRj	14	Vehicle	SC	QD	5 ml/kg	NA
3	DIO-NASH	Male	C57BL/6JRj	14	Chow-reversal + Vehicle	SC	QD	5 ml/kg	NA

Improvement in metabolic and biochemical parameters

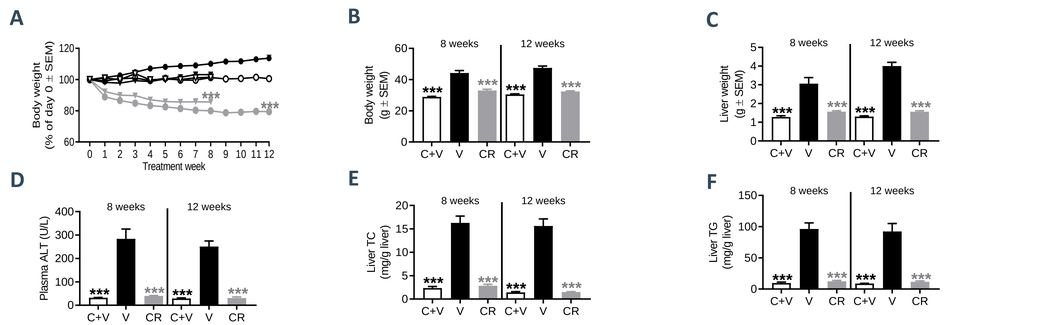


Figure 1. Chow reversal improves metabolic and biochemical parameters in GAN DIO-NASH mice. **(A)** Terminal body weight (g). **(B)** Terminal liver weight. **(C)** Terminal plasma alanine aminotransferase (ALT). **(D)** Terminal plasma aspartate aminotransferase (AST). **(E)** Terminal plasma triglycerides. **(F)** Terminal plasma total cholesterol. **(G)** Terminal liver total cholesterol. **(H)** Terminal liver triglycerides. ***p<0.001 compared to corresponding DIO-NASH vehicle control (Dunnett's test one-factor linear model).

Improvement in NAFLD Activity Score and Fibrosis Stage

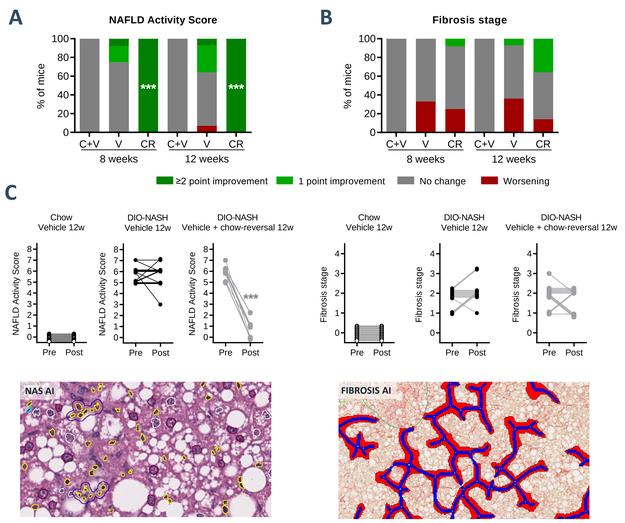


Figure 2. Chow-reversal improves liver histopathological scores in GAN DIO-NASH mice.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS and individual pre-post Fibrosis stage. ***p<0.001 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

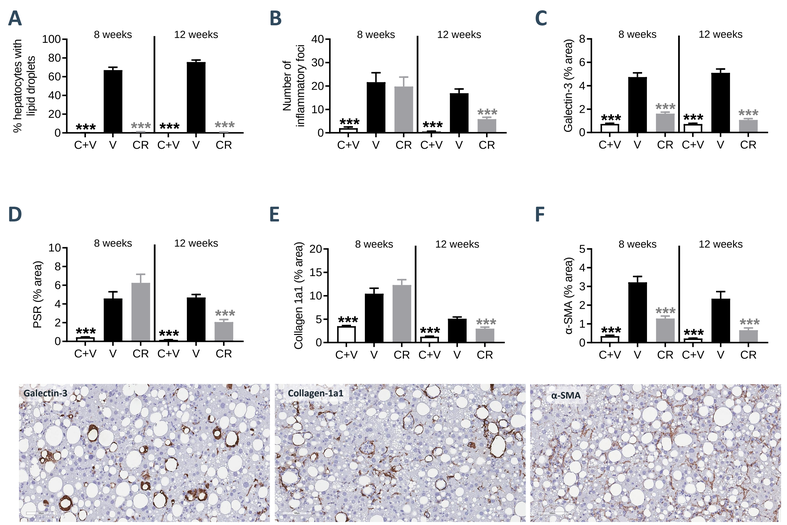


Figure 3. Chow-reversal improves quantitative liver histological markers in GAN DIO-NASH mice.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). (**A**) % hepatocytes 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) as marker for stellate cell activation. Mean ± SEM. ***p<0.001 to corresponding 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 transcriptomic profile for fibrosis

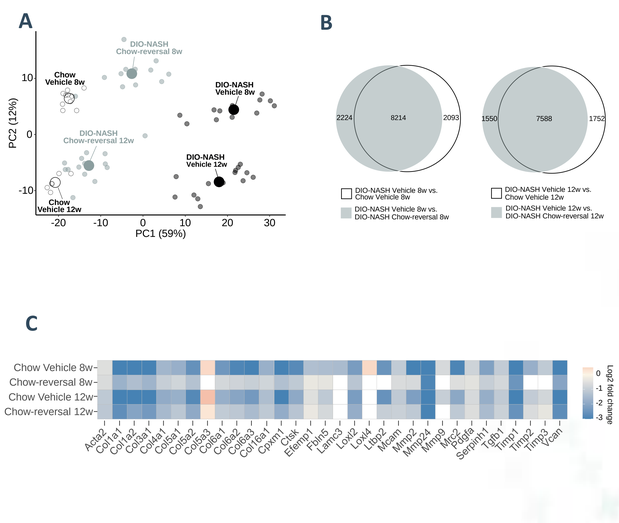


Figure 4. Chow-reversal suppress fibrosis-associated genes in GAN DIO-NASH mice.
(A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Venn diagram depicting shared and separate differentially expressed genes in treatment groups. **(C)** Regulation of hepatic extracellular matrix (ECM) candidate genes (log2-fold change compared to corresponding DIO-NASH vehicle control mice). Blue colour gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to corresponding vehicle-dosed DIO-NASH vehicle mice.

CONCLUSION

- Chow-reversal normalizes body weight, plasma ALT and liver lipids.
- Chow-reversal promotes ≥2-point significant improvement in NAFLD Activity Score.
- Fibrosis stage was unaffected by chow-reversal, illustrating highly stable collagen architecture
- Chow-reversal reduces quantitative histological markers of steatosis, inflammation and fibrosis
- Chow-reversal demonstrate transcriptomic suppression of fibrosis-associated gene expression.
- These findings agree with clinical findings, further highlighting clinical translatability of the GAN DIO-NASH mouse model