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

Authors
Kristoffer Voldum-Clausen, Denise Oro, Martin Rønn Madsen, Michael Feigh.

Gubra, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

BACKGROUND & AIM

Current standard of care for non-alcoholic steatohepatitis (NASH) involves lifestyle modification, notably dietary intervention, aiming to promote regression or resolution of NASH and liver fibrosis. The present study aimed to evaluate the metabolic, biochemical, histological and transcriptomic effects of 8-, 16- and 24-weeks dietary intervention (chow-reversal) in the Gubra-Amylin NASH (GAN) diet-induced obese (DIO) mouse model of fibrosing NASH.

1 Study outline

Group #	Group name	Number of Animals	Animal model	Dosing frequency	Route of administration
1	Chow Vehicle	10	LEAN-CHOW	QD	SC
2	Vehicle 8W	16	DIO-NASH	QD	SC
3	Reversal 8W	16	DIO-NASH	QD	SC
4	Vehicle 16W	16	DIO-NASH	QD	SC
5	Reversal 16W	14	DIO-NASH	QD	SC
6	Vehicle 24W	15	DIO-NASH	QD	SC
7	Reversal 24W	15	DIO-NASH	QD	SC

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

2 Improvement in metabolic and biochemical parameters

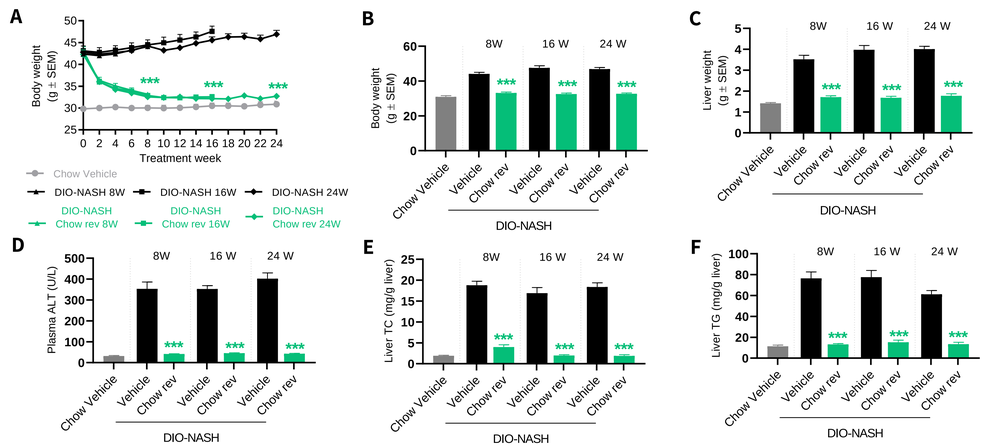

Figure 2. Dietary intervention improves metabolic and biochemical parameters in GAN DIO-NASH mice. **(A)** Absolute body weight during time. **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(D)** Terminal plasma aspartate aminotransferase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

3 Improvement in NAFLD Activity Score

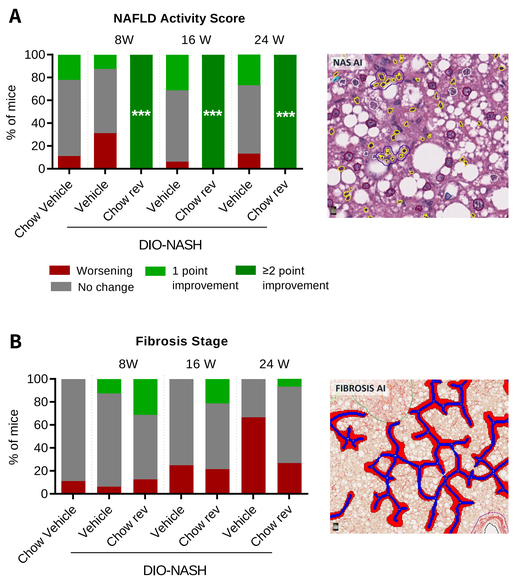

Figure 2. Dietary intervention improves histopathological NAFLD Activity Score in GAN DIO-NASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. ***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.

4 Improvement in quantitative histology of steatosis, inflammation and fibrogenesis

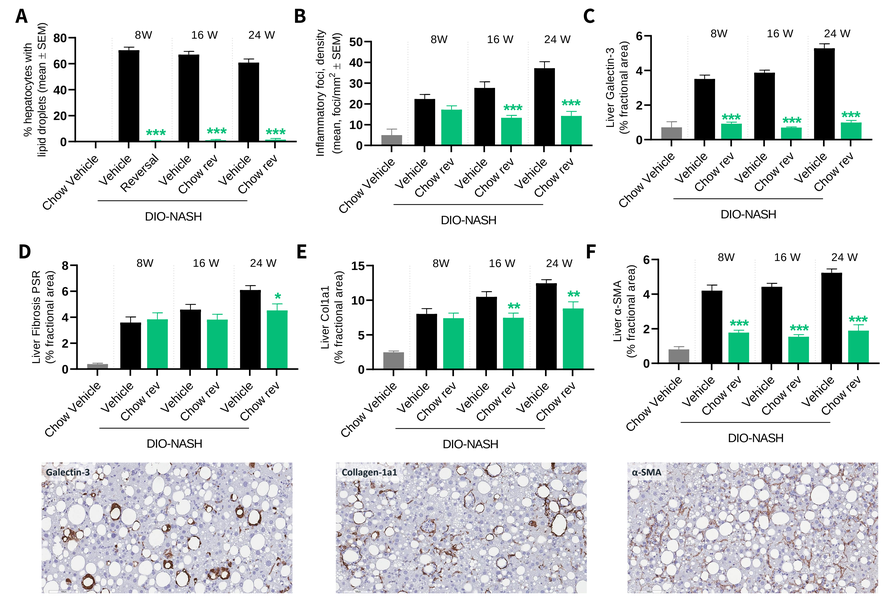

Figure 3. Dietary intervention improves quantitative liver histological markers in GAN DIO-NASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). (**A**) % hepatocytes 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<0.05, **p<0.01 ***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).

5 Improvement in transcriptomic profile for fibrosis and inflammation

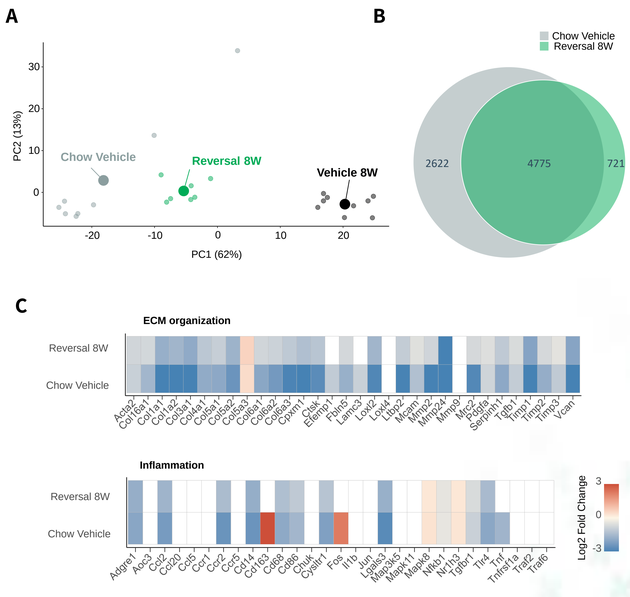

Figure 5. Dietary intervention suppress fibrosis- and inflammation-associated genes in GAN DIO-NASH mice. **(A)** Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Venn diagram depicting shared and separate differentially expressed genes in treatment groups. **(C)** Regulation of hepatic extracellular matrix (ECM) 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

+ Dietary intervention normalizes body weight, plasma ALT and liver lipids.
+ Dietary intervention promotes ≥2-point significant improvement in NAFLD Activity Score.
+ Fibrosis stage was unaffected by dietary intervention, illustrating highly stable collagen architecture.
+ Dietary intervention reduces quantitative histological markers of steatosis, inflammation, fibrosis and stellate cell activation.
+ Dietary intervention demonstrate transcriptomic suppression of fibrosis- and inflammation- associated gene expression already after 8 weeks.
+ These findings agree with clinical findings, further highlighting clinical translatability of the GAN DIO-NASH mouse model.

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