# Dietary intervention improves intestinal permeability and reduces colonic fibro-inflammation in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH


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## Background & Aim

The gut-liver axis is considered playing an important role in metabolic dysfunction-associated steatohepatitis (MASH). Accordingly, MASH is associated with gut dysbiosis and impaired intestinal barrier function ('leaky gut') which may expose the liver to inflammatory microbial products and facilitate pro-fibrotic responses. The Gubra-Amylin NASH (GAN) diet-induced obese (DIO) mouse is an industry-standard translational model of biopsy-confirmed MASH and fibrosis. The present study aimed to assess the impact of dietary intervention (chow reversal) on markers of intestinal permeability and hepatic/colonic inflammation and fibrosis in the biopsy-confirmed GAN DIO-MASH mouse.

## Methods

Male C57BL/6JRj mice were fed the GAN diet for 38 weeks and mice with liver biopsy-confirmed MASH (NAS≥5) and fibrosis (stage F2-F3) were included in the study. GAN DIO-MASH mice received chow-reversal for 12 weeks (n=14). Vehicle-dosed GAN DIO-MASH mice remaining on the GAN diet served as controls (n=13). Age-matched chow-fed animals were included as healthy controls (n=8). Within-subject comparisons (pre-post) were performed for histopathological scores (NAS and fibrosis stage) using Gubra Histopathological Objective Scoring Technique (GHOST). Intestinal permeability testing (FITC-dextran). Terminal endpoints included quantitative histological markers of liver and colon inflammation (CD45) and fibrosis (PSR) and intestinal markers of tight junction proteins (ZO-1, occludin).

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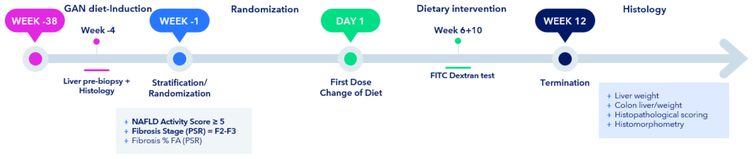
## 1 Study outline




| Group | Animal | Gender | Number of animals | Treatment | Administration r oute | Dosing frequency |
|---|---|---|---|---|---|---|
| 1 | LEAN-CHOW | Male | 8 | Vehicle | PO | QD |
| 2 | DIO-MASH | Male | 13 | Vehicle | PO | QD |
| 3 | DIO-MASH | Male | 14 | Vehicle + Chow reversal | PO | QD |

**Figure 1. Study outline.** Abbreviations: PO; per oral, QD; once daily, NA; not applicable, GAN; Gubra Amylin NASH.

## 2 Dietary intervention improves metabolic parameters and MASH

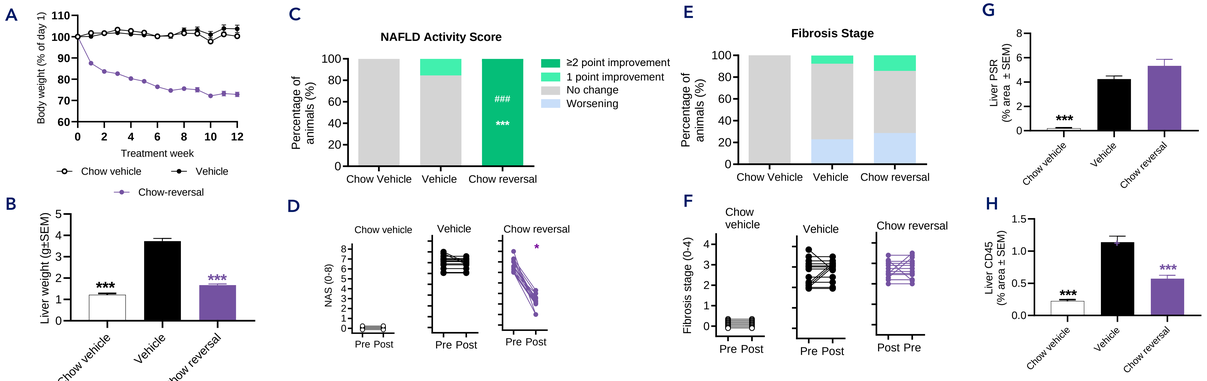



**Figure 2. Dietary intervention improves body weight, liver weight and MASH.** **(A)** Relative body weight during study period. **(B)** Terminal liver weight. **(C-F)** Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(C)** NAFLD Activity Score (NAS). ***p<0.001, and ### p<0.001 compared to vehicle (One-sided Fisher's exact test). **(D)** Individual pre-post NAS. **(E)** Fibrosis score. **(F)** Individual pre-post fibrosis stage. **(G-H)** Quantitative histological markers of fibrosis and inflammation **(G)** % area of PSR **(H)** % area of CD45. ***p<0.001 compared to vehicle.

## 3 Dietary intervention improves intestinal permeability and colonic fibro-inflammation

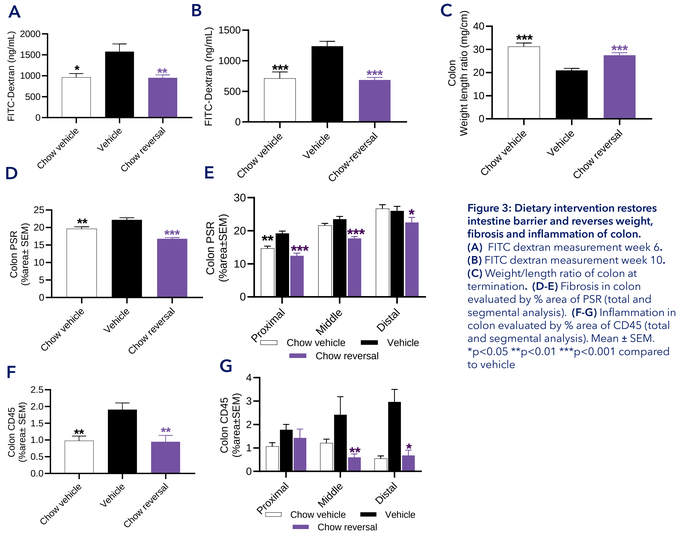



**Figure 3: Dietary intervention restores intestine barrier and reverses weight, fibrosis and inflammation of colon.** **(A)** FITC dextran measurement week 6. **(B)** FITC dextran measurement week 10. **(C)** Weight/length ratio of colon at termination. **(D-E)** Fibrosis in colon evaluated by % area of PSR (total and segmental analysis). **(F-G)** Inflammation in colon evaluated by % area of CD45 (total and segmental analysis). Mean ± SEM. *p<0.05 **p<0.01 ***p<0.001 compared to vehicle

## 4 Dietary intervention increases intestinal tight junction protein

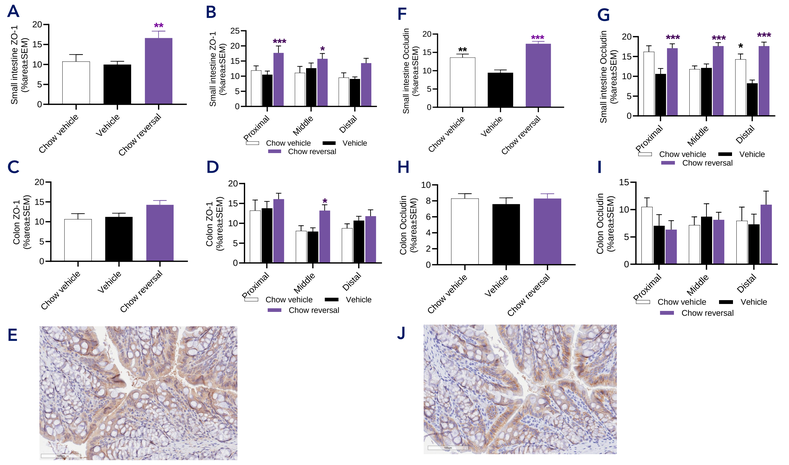



**Figure 4. Dietary intervention increases the expression of gut tight junction proteins.** **(A-B)** % area of ZO-1 in small intestine (total and segmented). **(C-D)** % area of ZO-1 in colon (total and segmented). **(E)** Representative photomicrographs of ZO-1 in colon. **(F-G)** % area of occludin in the small intestine (total and segmented). **(H-I)** % area of occludin (total and segmented). **(J)** Representative photomicrographs of occludin in the colon. *p<0.05, **p<0.01, ***p<0.001 compared to vehicle.

## Conclusion

- The GAN DIO-MASH mouse shows increased intestinal permeability, colonic hypotrophy and fibro-inflammation
- Dietary intervention markedly improves metabolic and liver histological hallmarks of MASH but has no effect on liver fibrosis scores
- Dietary intervention improves intestinal permeability, increases intestinal tight junction protein expression and reduces colonic fibro-inflammation.
- The benefits of dietary intervention on intestinal permeability markers further supports clinical translatability of the biopsy-confirmed GAN DIO-MASH mouse model

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