Novel Gubra Amylin NASH (GAN) diet-induced obese mouse models of biopsy-confirmed non-alcoholic steatohepatitis

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INTRODUCTION AND AIM

The Amylin Liver NASH (AMLN) diet-based *ob/ob* and C57BL/6J mouse models display clinical translatability with respect to key metabolic and liver biopsy-confirmed pathological changes associated with non-alcoholic steatohepatitis (NASH). A recent FDA ban on trans-fats in foods has prompted the development of a new NASH diet capable of promoting a compatible level of disease, as the AMLN diet contains trans-fat-containing Primex shortening. The present study aimed to assess the metabolic and liver pathological phenotype in *ob/ob and* C57BL/6J mice fed a palmitic acid-enriched high-fat diet with a nutrient composition and caloric content similar to the AMLN diet.

METHODS

Male *ob/ob* mice were fed chow, AMLN diet (40% total fat kcal of which 18.5% were trans-fat kcal, 20% fructose, 2% cholesterol; Research Diets #D09100301) or a modified AMLN diet with Primex substituted by equivalent amounts of palm oil (Research Diets, #D09100310), termed Gubra Amylin NASH (GAN) diet, for up to 30 weeks. C57BL/6J mice were fed the same diets for 28 weeks. NAFLD activity score (NAS) and fibrosis staging was assessed. Quantitative histomorphometric analyses included fractional (%) area of steatosis (hematoxylin-eosin), inflammation (galectin-3), and collagen (Col1a1). RNA sequencing was performed on terminal liver samples.

RESULTS

Weight gain, body composition and liver mass in *ob/ob*-NASH mice fed AMLN or GAN diet

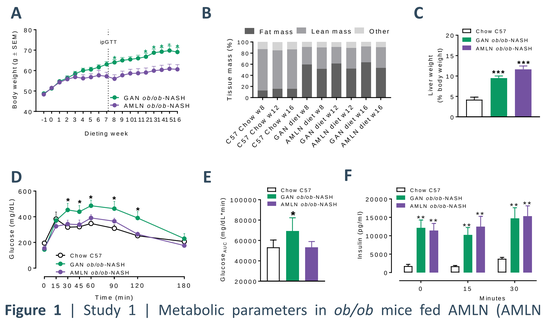

Figure 1 | Study 1 | Metabolic parameters in *ob/ob* mice fed AMLN (AMLN *ob/ob*-NASH) or GAN (GAN *ob/ob*-NASH) diet for 16 weeks. A) Body weight gain, B) body composition, C) terminal liver weight (week 16), D) ipGTT, E) $AUC_{glucose}$ (0-180 min), F) plasma insulin (0, 15, 30 min). ipGTT was performed in week 7 of the dieting period. *p<0.05, **p<0.01, ***p<0.001 vs. chow-fed C57BL/6J (chow C57) control mice.

Plasma and liver biomarker changes in *ob/ob*-NASH mice fed AMLN or GAN diet

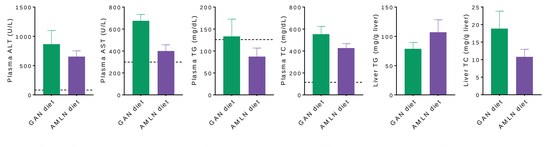

Figure 2 | Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglycerides (TG), total cholesterol (TC) and liver lipids (TG, TC) in *ob/ob* mice fed AMLN or GAN diet for 16 weeks. Horizontal dotted line indicates corresponding level in age-matched chow-fed C57BL/6J mice.

Comparable biopsy-confirmed NALFD Activity Scores in *ob/ob*-NASH mice fed AMLN or GAN diet

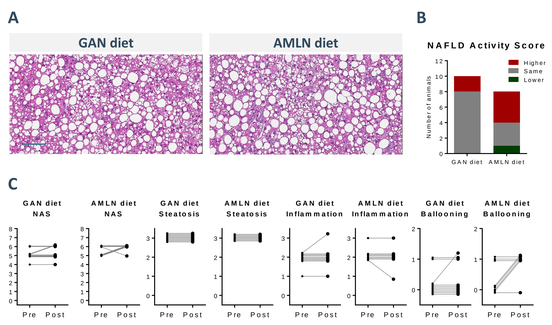

Figure 3 | A) Representative images of terminal liver morphology (HE staining, 20x magnification, scale bar 100 µm). B) Composite NAFLD Activity Score (NAS, number of animals with higher, same or lower post-biopsy score compared to pre-biopsy). C) Individual NAS, steatosis, inflammation and ballooning scores. Paired pre- and post-biopsies were samples at 9 and 16 weeks of feeding, respectively.

Comparable biopsy-confirmed fibrosis scores in *ob/ob*-NASH mice fed AMLN or GAN diet

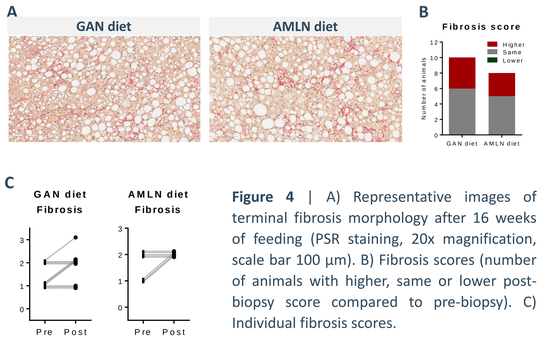

Figure 4 | A) Representative images of terminal fibrosis morphology after 16 weeks of feeding (PSR staining, 20x magnification, scale bar 100 µm). B) Fibrosis scores (number of animals with higher, same or lower post-biopsy score compared to pre-biopsy). C) Individual fibrosis scores.

Comparable quantitative histopathological changes in *ob/ob*-NASH mice fed AMLN or GAN diet

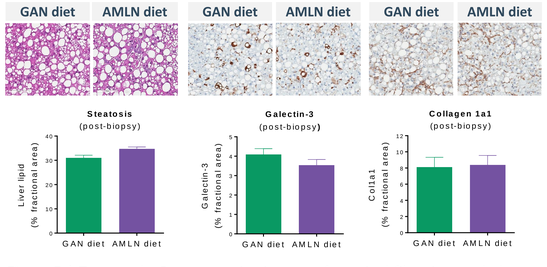

Figure 5 | Top panels: Representative images of terminal fibrosis morphology after 16 weeks of feeding. Lower panels: Fractional (%) area of steatosis (HE staining), inflammation (Galectin-3 IHC) and fibrosis (Col1a1 IHC) determined by imaging-based histomorphometry. Scale bar 100 µm.

Comparable liver transcriptome changes in *ob/ob*-NASH mice fed AMLN or GAN diet

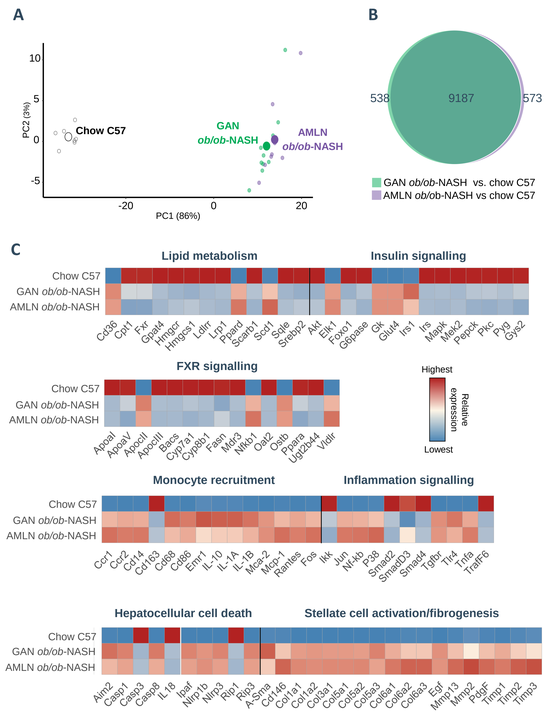

Figure 6 | RNA sequencing. Hepatic gene expression profiles in chow-fed C57BL/6J mice (Chow C57), AMLN and GAN *ob/ob*-NASH mice after 16 weeks of feeding. A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. B) Group-wise comparison of the total number of differentially expressed genes between GAN and AMLN *ob/ob*-NASH mice vs. chow-fed C57BL/6J mice. C) Relative gene expression levels (z-scores) of differentially regulated candidate genes associated with NASH and fibrosis.

High rates of bridging fibrosis in *ob/ob*-NASH mice fed AMLN or GAN diet for extended periods

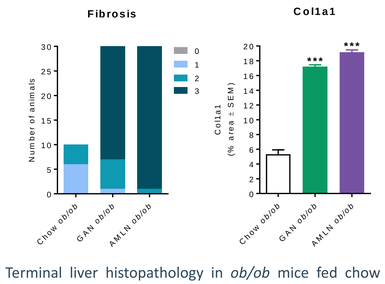

Figure 7 Terminal liver histopathology in *ob/ob* mice fed chow (n=10 mice), AMLN (AMLN DIO-NASH, n=30 mice) or GAN (GAN DIO-NASH, n=30 mice) diet for 30 weeks. Left panel, histopathological scoring of fibrosis. Right panel, fractional (%) area of collagen-1a1. ***p<0.001 vs. chow-fed *ob/ob* mice.

Comparable liver histopathology in DIO-NASH mice fed AMLN or GAN diet

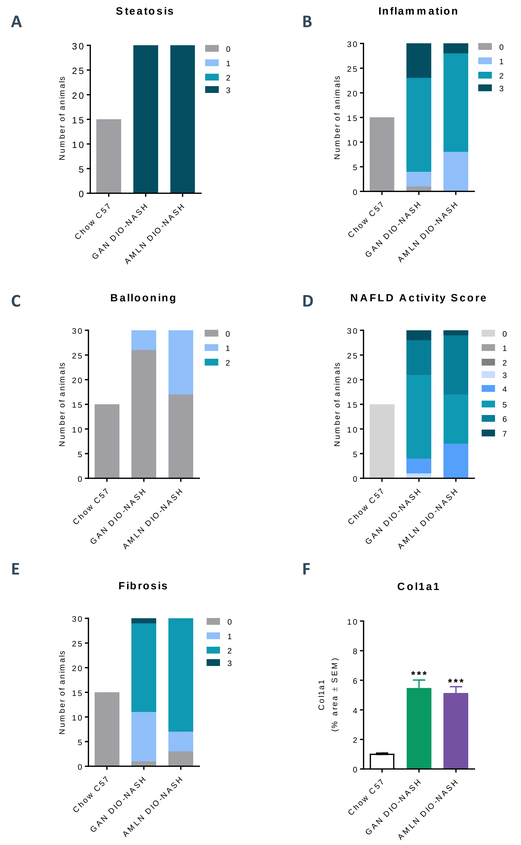

Figure 8 | Terminal liver histopathology in C57BL/6J mice fed chow (n=15 mice), AMLN (AMLN DIO-NASH, n=30 mice) or GAN (GAN DIO-NASH, n=30 mice) diet for 28 weeks. Histopathological scores of steatosis (A), lobular inflammation (B), hepatocyte ballooning (C), composite NAFLD Activity Score (NAS, D), and fibrosis (E). Fractional (%) area of collagen-1a1 (F). ***p<0.001 vs. chow-fed C57BL/6J mice.

CONCLUSIONS

- **Modification of the AMLN diet by substitution of Primex shortening with palm oil (GAN diet) results in a maintained NASH phenotype in both *ob/ob*-NASH and DIO-NASH mice.**
- **Compared to the AMLN diet, the GAN diet promotes further body weight gain and impairs glucose intolerance in *ob/ob*-NASH mice.**
- **The clear metabolic and histopathological hallmarks of fibrotic NASH in *ob/ob*-NASH and DIO-NASH mice fed the GAN diet highlight the suitability of this model for characterizing novel drug therapies for NASH.**