INTRODUCTION AND AIM

The Amylin Liver NASH (AMLN) diet-based ob/ob and C57BL/6 mouse models display clinical translatablenes with respect to key metabolic and liver pathophysiological changes as well as NASH. The recent FDA ban on trans-fats in foods has prompted the development of a new AMLN diet capable of promoting a detectable degree of liver disease, as the AMLN diet contains trans-fat-containing Primex shortening. The present study aimed to assess the metabolic and liver pathophysiological differences in ob/ob and C57BL/6 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, Primex shortening), or a modified AMLN diet with Primex substituted by equivalent amounts of palm oil (Research Diets, D100130S), termed Gubra Amylin NASH (GAN) diet, for up to 30 weeks. C57BL/6 mice were fed the same diet for 28 weeks. NASH activity score (NAS) and fibrosis staging was assessed. Quantitative histomorphometric analyses included fractional (F) area of steatosis (hematoxylin-eosin), inflammation (Galexin-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

Comparable biopsies-confined NALFD Activity Scores in ob/ob-NASH mice fed AMLN or GAN diet

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

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

CONCLUSIONS

- Modulation of the AMLN diet by substituting 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 improves 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.

Nutritional and Metabolic Parameters in ob/ob mice fed AMLN (AMLN diet) or GAN (GAN diet) for 16 weeks. A) Body weight gain, B) Body composition, C) Terminal liver weight (week 16). D) i.p. TTR. E) ALT. F) Plasma insulin (0, 15, 30 min), p<0.001 was performed in week 15 of the dieting period. *p<0.05, **p<0.01, ***p<0.001 vs. chow-fed C57BL/6 (Chow C57) control mice.

Steatohepatitis and steatosis development in ob/ob mice fed AMLN or GAN diet for 16 weeks. A) Body weight gain, B) Body composition, C) Terminal liver weight (week 16). D) i.p. TTR. E) ALT. F) Plasma insulin (0, 15, 30 min), p<0.001 was performed in week 15 of the dieting period. *p<0.05, **p<0.01, ***p<0.001 vs. chow-fed C57BL/6 (Chow C57) control mice.

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

Comparable quantitative histopathological changes in ob/ob-NASH mice fed AMLN or GAN diet for extended periods

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

Terminal liver histopathology in ob/ob-NASH 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 NALFD Activity Score (NAS; E), and fibrosis (F). Fractional (%) area of collagen-1a1. *p<0.001 vs. chow-fed C57BL/6(ob/ob) mice.

Figure 1 | Study 1 | Nutritional and metabolic parameters in ob/ob mice fed AMLN (AMLN diet) or GAN (GAN diet) for 16 weeks. A) Body weight gain, B) Body composition, C) Terminal liver weight (week 16). D) i.p. TTR. E) ALT. F) Plasma insulin (0, 15, 30 min), p<0.001 was performed in week 15 of the dieting period. *p<0.05, **p<0.01, ***p<0.001 vs. chow-fed C57BL/6 (Chow C57) control mice.

Figure 2 | Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglycerides (TG), total cholesterol (TC) and liver lipids (TL; 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/6 mice.

Figure 3 | Representative images of terminal liver morphology (H&E staining, 20x magnification, scale bar 100 µm). A) Composite NALFD 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 sampled at 5 and 16 weeks of feeding, respectively.

Figure 4 | A) Representative images of terminal liver morphology after 16 weeks of feeding (PSI 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.

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

Figure 6 | RNA sequencing. Hepatic gene expression profiles in chow-fed C57BL/6 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/6 mice. C) Relative gene expression levels (J-scores) of differentially regulated candidate genes associated with NAS and fibrosis.

Figure 7 | Terminal liver histopathology in ob/ob-NASH mice fed chow (n=15 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 C57BL/6(ob/ob) mice.

Figure 8 | Terminal liver histopathology in C57BL/6 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 NALFD Activity Score (NAS; E), and fibrosis (F). Fractional (%) area of collagen-1a1. **p<0.001 vs. chow-fed C57BL/6( ob/ob) mice.