Hepatocellular senescence and mitochondrial dysfunction in the GAN diet-induced obese mouse model of NASH

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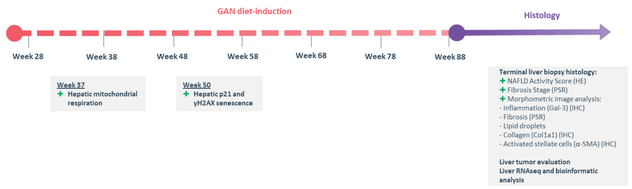
Background & Aim

The prevalence of obesity-associated non-alcoholic steatohepatitis (NASH) with development of advanced hepatic fibrosis and hepatocellular carcinoma (HCC) increases with age. This study aimed to characterize key markers of hepatocellular senescence and mitochondrial respiratory capacity in relation to disease progression in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH.

Study outline



Age and GAN diet-induced disease progression

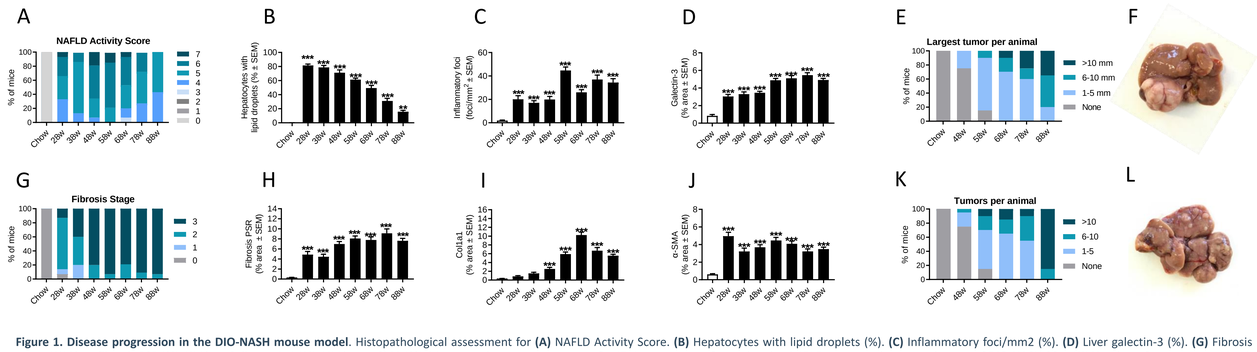


Figure 1. Disease progression in the DIO-NASH mouse model. Histopathological assessment for **(A)** NAFLD Activity Score. **(B)** Hepatocytes with lipid droplets (%). **(C)** Inflammatory foci/mm2 (%). **(D)** Liver galectin-3 (%). **(G)** Fibrosis stage. **(H)** Liver fibrosis PSR (%). **(I)** Liver Collagen 1a1 (%). **(J)** Liver α-SMA (%). Macroscopic tumor assessment for **(E)** Largest tumor for each animal. **(F)** Representative tumor images of DIO-NASH 88w. **(K)** Number of tumors per animal. **(L)** Representative tumor images of DIO-NASH 88w. **p<0.01, ***p<0.001 compared to Chow (Dunnett's test one-factor linear model).

Increased markers of hepatocellular senescence

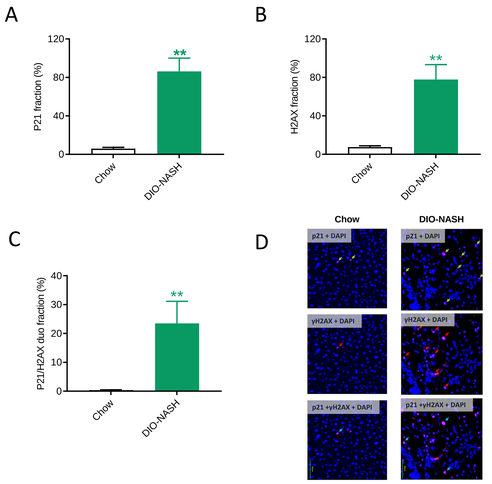


Figure 2. Increased p21 and γH2AX hepatocellular senescence markers in DIO-NASH mice. Fraction of **(A)** p21 positive cells, **(B)** γH2AX positive cells, and **(C)** Duo fraction of p21/γH2AX positive cells. **(D)** Representative FIHC images of DIO-NASH livers stained with p21 antibody, γH2AX antibody and DAPI. p21 positive cells are indicated by green arrows, H2AX positive cells by red arrows, and duo-positive cells by blue arrows. **p<0.01 compared to Chow (Dunnett's test one-factor linear model)

Compromised hepatocellular mitochondrial respiratory capacity

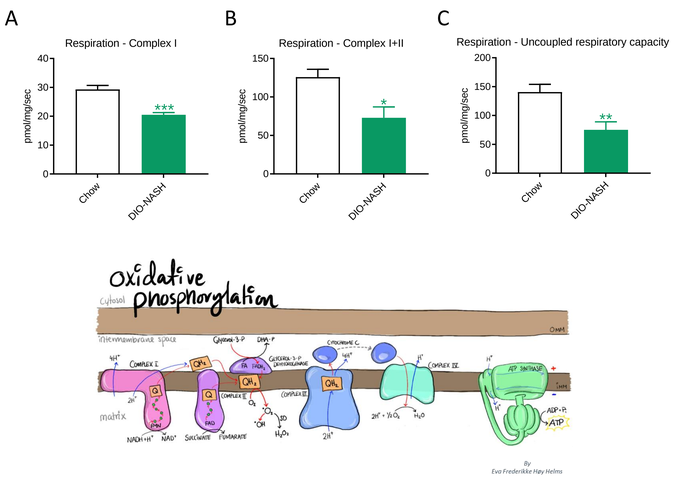


Figure 3. Compromised hepatocellular mitochondrial respiratory capacity in DIO-NASH mice. High-resolution respirometry assessment of **(A)** Complex I, **(B)** Complex I + Complex II, and **(C)** uncoupled respiratory capacity. *p<0.05, **p<0.01, ***p<0.001 compared to Chow (Dunnett's test one-factor linear model)

Transcriptomic profile for hepatocellular senescence

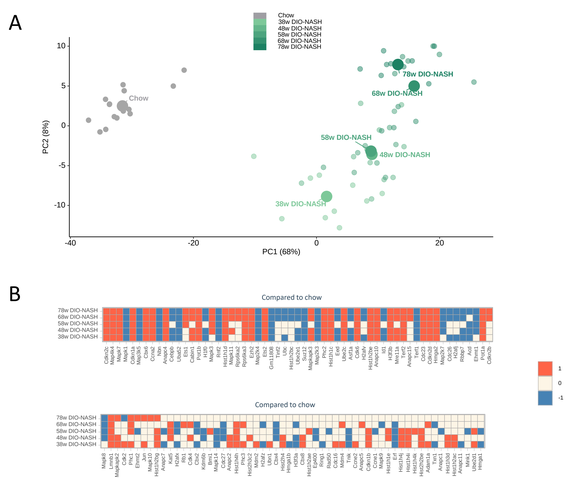


Figure 4. Increased hepatocellular senescence-associated genes in DIO-NASH mice. **(A)** Principal component analysis of the 500 most variable genes. **(B)** Heatmap of senescence-associated genes derived from the Reactome Pathway "Cellular Senescence". Blue and red colour gradients indicate the log2FC of significantly (p<0.05) down- and up-regulated gene expression, respectively. White boxes indicate genes not significantly (p>0.05) regulated.

CONCLUSION

- Aged DIO-NASH mice demonstrated clinical NAFLD Activity Score (≥4) after ≥28 weeks on GAN diet.
- Aged DIO-NASH mice progressed to bridging fibrosis (stage F3) after ≥48 weeks on GAN diet.
- Aged DIO-NASH mice developed tumors including HCC after ≥58 weeks on GAN diet.
- Aged DIO-NASH mice demonstrated increased histological markers of hepatocellular senescence.
- Aged DIO-NASH mice demonstrated compromised hepatocellular mitochondrial respiratory capacity.
- Aged DIO-NASH mice demonstrated hepatic senescence-associated gene expression signatures.
- The aged GAN DIO-NASH mice display features of accelerated hepatocellular senescence including impaired mitochondrial respiration, highlighting the utility of this model for profiling and targeting novel senescence-associated drug therapies.