

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic complication of obesity and metabolic syndrome and now the most common chronic liver disease in the world. As many as 116 million people in the EU alone may suffer from some form of NAFLD. It ranges from **simple steatosis**, then **NASH** to cirrhosis. Pathologists assess NASH severity using semiquantitative scoring methods, such as NAS or SAF score, which are linked to an inherent high variability. Histological scoring assessment provides discrete and not continuous values thus small variations may not translate into a different score.

## AIM

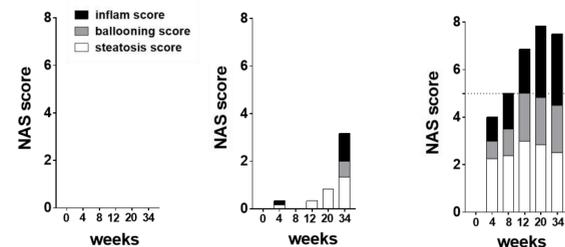
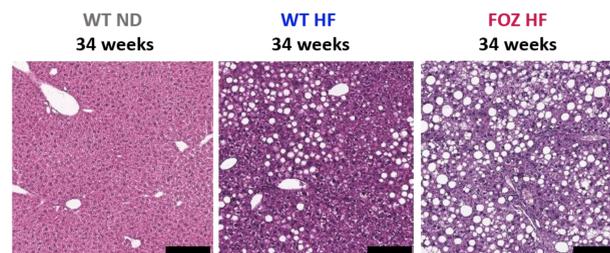
To perform a numerical quantification of NASH features, fully automatic, which is of particular interest for accurately monitoring evolution or regression upon therapy in pre-clinical studies.

## METHOD

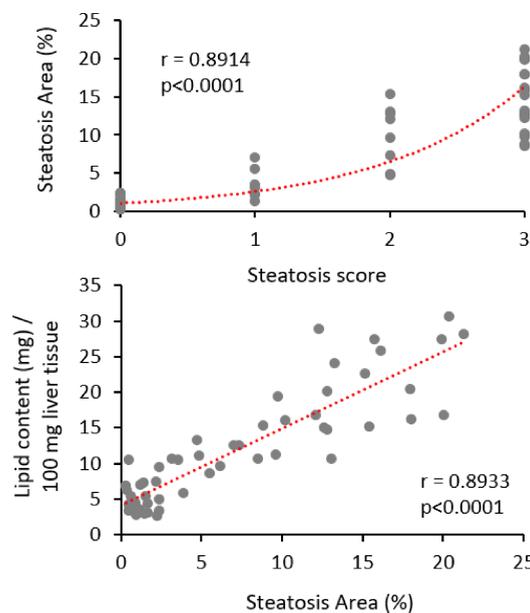
We followed **high fat diet-fed foz/foz mice (FOZ HF)**, known to develop progressive NASH over 34 weeks, which were compared to **high fat diet-fed wild type mice (WT HF)** and normal diet-fed wild type mice. Automated software image analysis of steatosis, inflammation and fibrosis was performed on digital images from entire liver sections stained for the respective purposes: **H&E for steatosis**, immunostaining of **F4/80 for inflammation** and **picrosirius red for fibrosis**. Data obtained from numerical analysis were compared with NAS score, biochemical quantification and gene expression.

## RESULTS

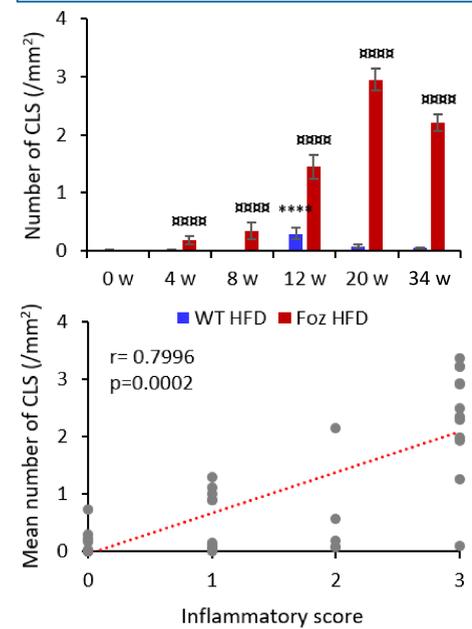
**FOZ HF had histologically proven NASH** after 8 to 12 weeks of high fat diet with NAS score  $\geq 5$  while **WT HF mice exhibited simple steatosis** until late timepoints.



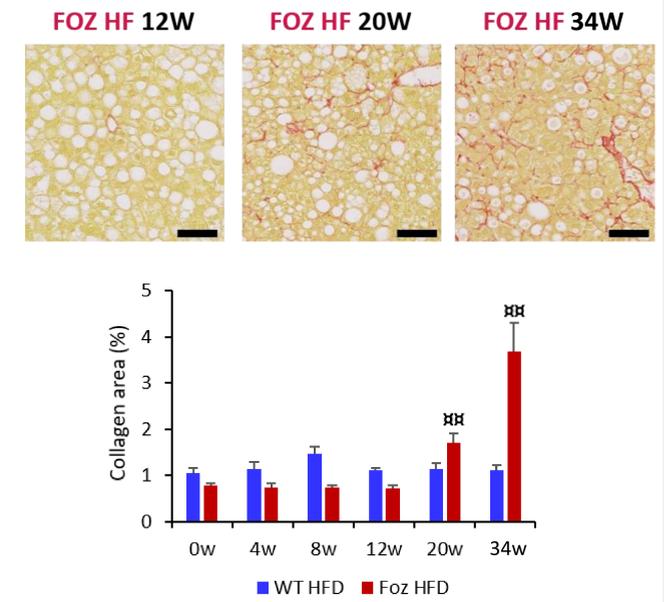
**Steatosis area** generated by automated digital analysis from **H&E** stained liver sections correlates with histological scoring and biochemical analysis.



**Quantification of crown-like structures** by automated digital analysis from **anti-F4/80** stained liver sections shows a rapid increase in **FOZ HF** until 20W and correlates with histological scoring and gene expression.



**Quantification of collagen deposits** by automated digital analysis from **picrosirius red** stained liver sections shows an increase of collagen in **FOZ HF** starting at 20W, consistent with qPCR results (collagen 1 $\alpha$ 1, MMP13 and TIMP1).



## CONCLUSIONS

Our results show remarkable correlations between digital analysis-based data's and NAS score/ biochemical quantification/ gene expression. Therefore, we can precisely and easily quantify key NASH features such as **steatosis, inflammation and fibrosis**, although ballooning remains a big challenge. Software-based fully-automated NASH method represents a promising accurate and reliable quantitative analysis to rapidly monitor disease activity with high-throughput in large pre-clinical studies.

## REFERENCES

- Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease, Juan Pablo Arab, Marco Arrese, Michael Trauner, Annual Review of Pathology: Mechanisms of Disease 2018 13:1, 321-350.
- Design and validation of a histological scoring system for nonalcoholic fatty liver disease, Kleiner DE et al., Hepatology, 2005 Jun;41(6):1313-21.

## REMARKS

- \* = Comparison to **WT HF** at week 0
- ⊠ = Comparison to **Foz HF** at week 0
- Data's are shown as mean  $\pm$  SD
- r = Spearman coefficient

## CONTACT INFORMATION

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