Automated shear wave elastography captures MASLD onset in C57BL/6NTac mice preconditioned on a modified Amylin liver MASH diet

L. Griffin[1], S. Yeung[2], S. Radhakrishnan[2], C. J. Moore[3], J. D. Rojas[3], B. Velasco[4], P. A. Dayton[4], and T. J. Czernuszewicz[3,4]

[1]Taconic Biosciences, Inc., Rensselaer, NY, USA, [2]Research Diets, Inc., New Brunswick, NJ, USA, [3]Revvity, Inc., Durham, NC, USA, [4]University of North Carolina Chapel Hill, Chapel Hill, NC, USA

Scan this code to download this poster or connect with one of our scientists!

Background and Aims

- C57BL/6 mice fed a modified Amylin liver NASH (aka GAN) diet demonstrate SLD and more closely recapitulate human MASLD compared to other preclinical models.
- Despite superior translatability, inherent variability exists and can only be assessed via invasive procedures (liver biopsy). Induction timeframes to fibrosis are also lengthy (>30 weeks on diet)
- Non-invasive tools for humans exist, but these tools have not been entirely validated in preclinical research

Aims:

- To validate automated shear wave elastography as a tool to assess MASH and fibrosis onset in commercially available NASH B6 mice
- To assess variability in MASH onset in NASH B6 mice
- To assess whether repeated SWE imaging may induce stress responses in the model, leading to altered phenotype progression

Imaging Methods

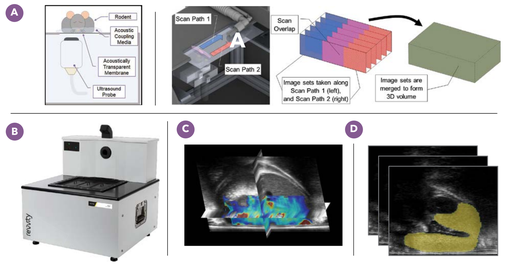


Figure 1. Schematic overview of image acquisition approach. **A:** Rodents are placed on the ultrasound instrument in prone position and imaged from below via robotically controlled raster scan. Raw 2D frames are reconstructed into 3D volumes. **B:** Photograph of Vega ultrasound *in vivo* imaging system. **C:** Screenshot of multi-modal 3D B-mode and SWE scan of a mouse liver in orthoslice view. **D:** Screenshot showing output of AI-assisted 3D liver segmentation (yellow outline). Segmentation is used to quantify liver volume, liver stiffness, and liver echogenicity.

Study Design

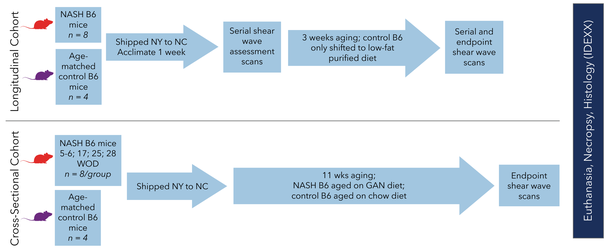


Figure 2. Overview of animal cohorts and experimental timeline. NASH B6 animals were shipped from Taconic Biosciences to UNC, where they were acclimated on respective diets and underwent SWE measurement at specific experimental timepoints.

Representative *In Vivo* Images

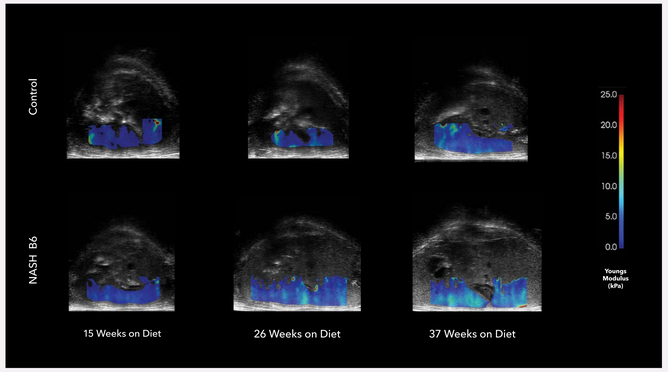


Figure 3. Representative images of mice on Control diet (top row) and NASH chow (bottom row). Anatomical B-mode images are shown in grayscale, while SWE stiffness maps are overlaid with blue-red colormap. Over time, NASH livers were significantly larger and brighter than controls, indicating hepatomegaly and steatosis. Liver stiffness in NASH livers was marginally higher, suggesting limited fibrosis.

Representative Histology

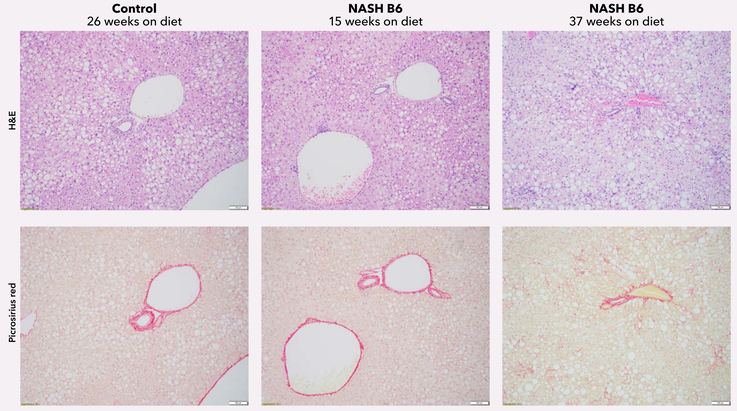


Figure 4: Representaitve images of H&E and PSR stains for control animals as well as NASH B6 animals after 15 and 37 weeks of diet conditioning.

Longitudinal *In Vivo* Measurements

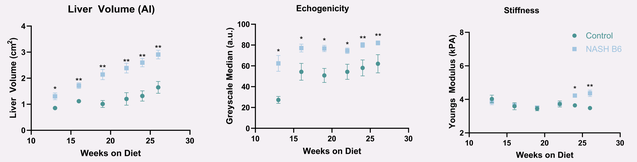
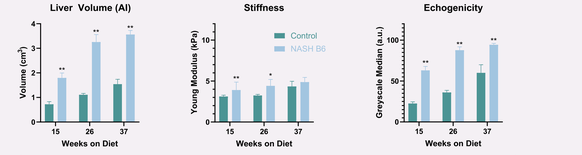


Figure 5: Liver volume, echogenicity, and stiffness measurements quantified from *in vivo* images over time. Both liver volume and echogenicity were significantly higher in NASH cohort compared to controls from first timepoint at 13 weeks, and increased considerably over time (2.2-fold and 1.3-fold for volume and echogenicity, respectively). Liver stiffness started to increase marginally after 24 WOD. Wilcoxon rank sum; * P≤0.05; ** P≤0.01.

Cross-Sectional *In Vivo* Measurements



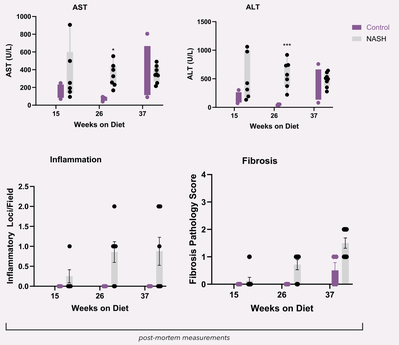


Figure 6: Liver volume, echogenicity, and stiffness measurements quantified in cross-sectional cohort. Nearly identical trends were observed as in longitudinal cohort; namely, volume and echogenicity were much higher than controls, while stiffness was marginally higher. Matched timepoints at 16 and 26 weeks between longitudinal and cross-sectional cohorts showed non-significant measurements across the board, suggesting no influence of serial imaging on biological progression of SLD. AST and ALT measurements across timepoints were not significantly different in most cases, nor were they indicative of phenotype progression. Pathologist scored inflammation and fibrosis indicate increasing trends over time compared with controls, though fibrosis failed to increase beyond F2 in most cases, despite 37 weeks of diet conditioning.

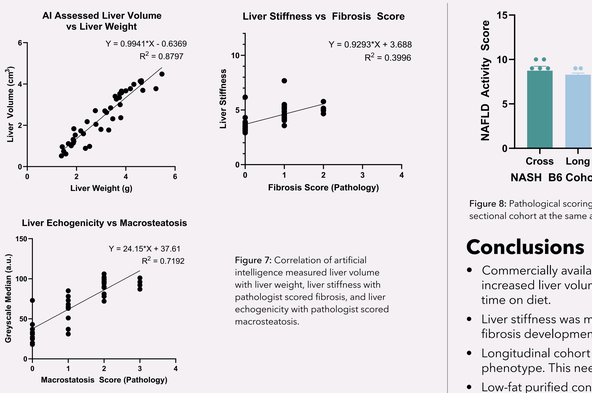


Figure 7: Correlation of artificial intelligence measured liver volume with liver weight, liver stiffness with pathologist scored fibrosis, and liver echogenicity with pathologist scored macrosteatosis.

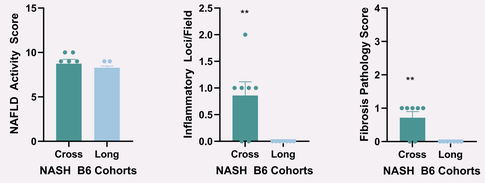


Figure 8: Pathological scoring of NAS, inflammatory loci, and fibrosis of the longitudinal cohort at 26 weeks on diet compared to a cross-sectional cohort at the same age indicated minor differences in phenotype severity. Unpaired t-test; * P≤0.05; ** P≤0.01.

Conclusions

- Commercially available NASH B6 mice from Taconic Biosciences demonstrated significantly increased liver volume and liver echogenicity, compared to age-matched controls, consistent with time on diet.
- Liver stiffness was marginally increased compared to age matched controls, suggesting limited fibrosis development.
- Longitudinal cohort demonstrated small but significant reduction in inflammation and fibrosis phenotype. This needs more research to understand.
- Low-fat purified control diets may induce liver steatosis, as indicated by increases in liver echogenicity over time.
- Vega may be a useful tool for screening and randomization of animals based on liver volume, steatosis onset, and fibrosis stage in preclinical drug testing.