Non-invasive staging of liver disease in C57BL/6NTac mice preconditioned on the modified-Amylin NASH diet using automated shear wave elastography

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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:

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

Imaging Methods

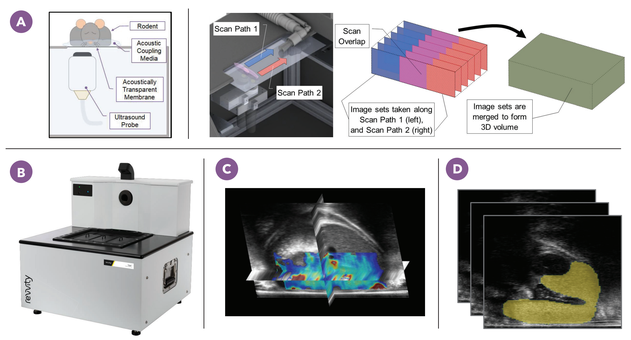

Figure 1. Schematic overview of image acquisition approach. **A:** Rodents are placed on the ultrasound instrument in prone position and imaged from below via robotically controlled raster scan. Raw 2D frames are reconstructed into 3D volumes. **B:** Photograph of Vega ultrasound *in vivo* imaging system. **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

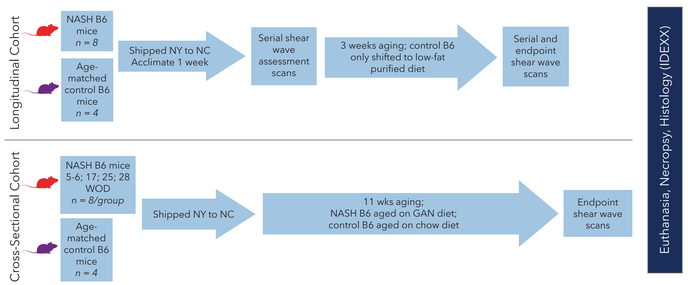

Figure 2. Overview of animal cohorts and experimental timeline. NASH B6 animals were shipped from Taconic Biosciences to UNC, where they were acclimated on respective diets and underwent SWE measurement at specific experimental timepoints.

Representative *In Vivo* Images

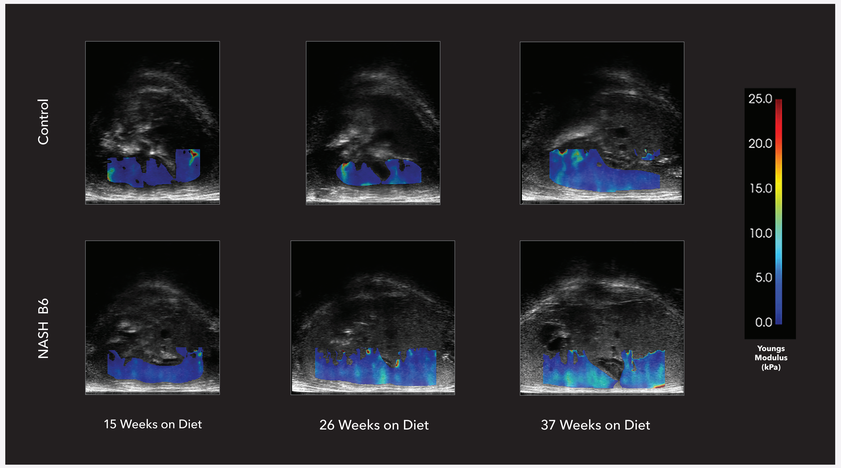

Figure 3. Representative images of mice on Control diet (top row) and NASH chow (bottom row). Anatomical B-mode images are shown in grayscale, while SWE stiffness maps are overlaid with blue-red colormap. Over time, NASH livers were significantly larger and brighter than controls, indicating hepatomegaly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

Longitudinal *In Vivo* Measurements

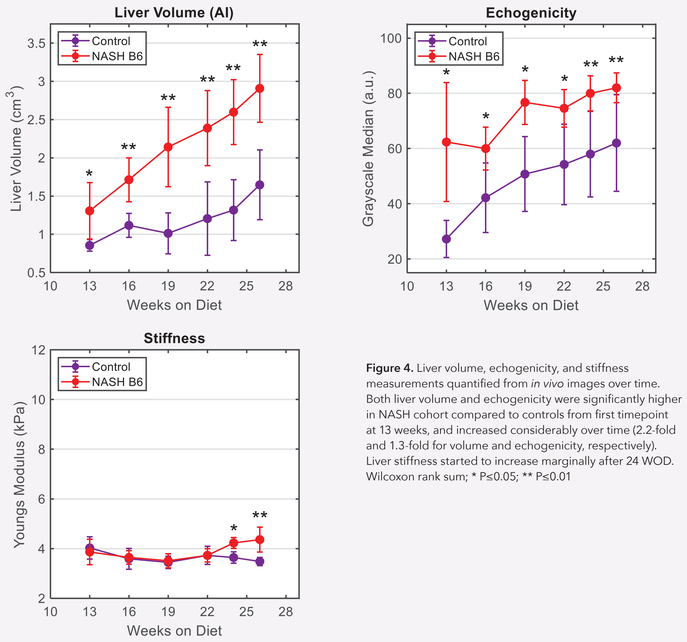

Figure 4. Liver volume, echogenicity, and stiffness measurements quantified from *in vivo* images over time. Both liver volume and echogenicity were significantly higher in NASH cohort compared to controls from first timepoint at 13 weeks, and increased considerably over time (2.2-fold and 1.3-fold for 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

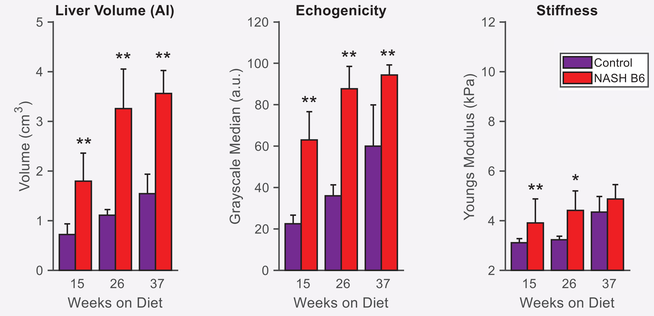

Figure 5. Liver volume, echogenicity, and stiffness measurements quantified in cross-sectional cohort. Nearly identical trends were observed as in longitudinal cohort; namely, volume and echogenicity were much higher than controls, while stiffness was marginally higher. Matched timepoints at 16 and 26 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.

AI-Assisted *In Vivo* vs. *Ex Vivo* Comparison

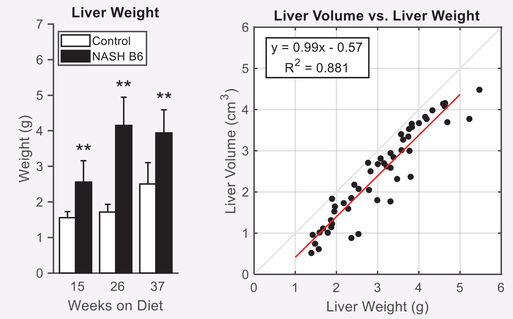

Figure 6. Bar plot (left) shows *ex vivo* liver weight for cross-sectional cohort, and scatter plot (right) shows comparison of *in vivo* liver volume to *ex vivo* liver weight. *In vivo* measurements consistently underestimated ground truth *ex vivo* values by ~0.5 cm^3, however strong correlation (R2 = 0.88) was observed despite this bias.

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.
- Repeated Vega measurements did not significantly alter phenotype progression in NASH B6 mice.
- 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.