In vivo multimodal imaging of NASH disease progression in a mouse model

Jen-Chieh Tseng[1], Arin Stowman[1], Alexis Stanley[1], Tomek Czernuszewicz[2], Ryan Gessner[2], Jeffrey D. Peterson[1]

[1]**PerkinElmer, Inc., Hopkinton, MA, USA**
[2]**SonoVol, Inc., Durham, NC, USA**

Abstract

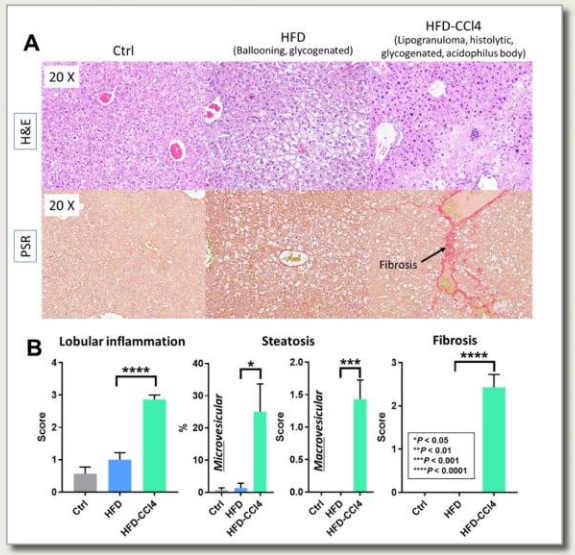
Non-alcoholic steatohepatitis (NASH), the most common chronic liver condition in western populations, is characterized by tissue damage and inflammation which leads to buildup of fat and fibrosis in the liver. We test if *in vivo* imaging techniques can be used to detect, visualize, and quantify disease progression in a mouse NASH model. The model is established by repeated i.p. injections of carbon tetrachloride (CCl4, mixed in corn oil) to female hairless SKH-1 mice, with a total weekly dose at 0.4 ml/kg. CCl4 is known to cause liver inflammation, and long-term exposure of CCl4 in conjunction with high fat diet (HFD) leads to liver steatosis and fibrosis.

Three types of imaging modalities were tested in this study. (1) **Fluorescence (FLI):** 2D planar and 3D tomography imaging to detect liver inflammation using specific IVISense™ inflammation probes (IV680 and AMT750, PerkinElmer Inc.); (2) **Ultrasound:** shear wave elastography (SWE) to detect changes in liver stiffness; (3) **MicroCT:** HU assessment to measure liver density changes associated with steatosis and/or fibrosis.

Three groups of mice were used in this study. (1) **Ctrl** control mice received saline injections and were maintained on regular diet; (2) **HFD** mice received saline injections but were maintained on high fat diet (Teklad custom diet TD.02028, Envigo); (3) **HFD-CCl4** mice received CCl4 injections and were maintained on high fat diet for NASH development.

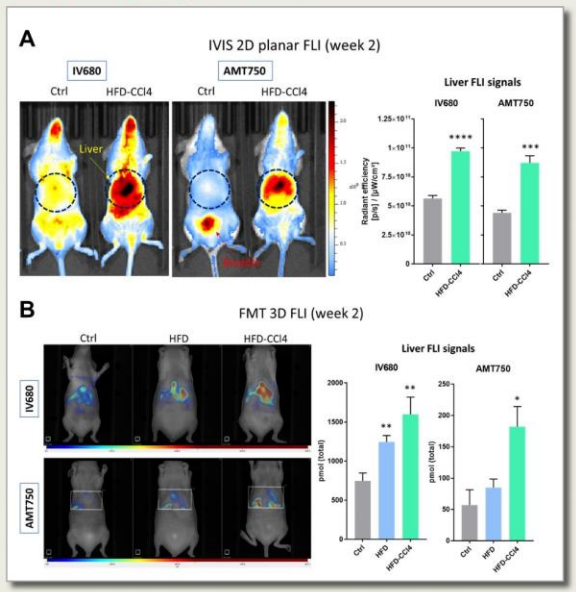
Fluorescence imaging can detect early liver inflammation in HFD-CCl4 livers in two weeks of NASH development. SWE imaging showed that, three weeks after CCl4 induction, the HFD-CCl4 livers started to develop lower liver stiffness, indicating fat accumulation and steatosis. Interestingly, this phenomenon reversed after the 8th week, and HFD-CCl4 livers became stiffer, suggesting extensive tissue fibrosis. The microCT HU assessment of liver density in the NASH mice also supports the SWE findings. The HFD-CCl4 livers showed lower HU readings at week 3. However, at week 12, HFD-CCl4 livers showed recovery in HU, suggesting development of denser fibrosis tissues. Our results support the use this multimodal strategy to non-invasively visualize and quantitatively analyze of NASH disease progression in animal models.

1 Histological validation of NASH development in the HFD-CCl4 livers



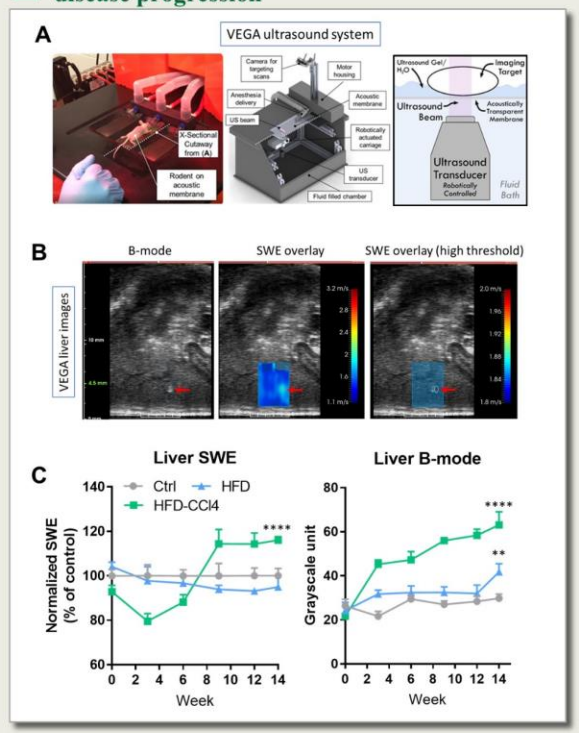
Histological validation of livers harvested after 7 weeks of disease development. (**A**) Top panels show H&E staining of liver sections of Ctrl, HFD and HFD-CCl4 mice. The HFD cause livers to develop ballooning and glycogenated hepatocytes. In the HFD-CCL4 livers, in addition to glycogenation, acidophilic and histolytic areas and lipogranuloma indicate extensive inflammation and fat accumulation. Lower panels show positive PSR staining of fibrotic regions only in the HFD-CCl4 livers. (**B**) Quantitative representation of histological scoring of NASH features. Only the HFD-CCl4 mice developed all three major pathological characteristics of NASH: inflammation, steatosis, and fibrosis.

2 Fluorescence imaging of liver inflammation at the early stage of NASH



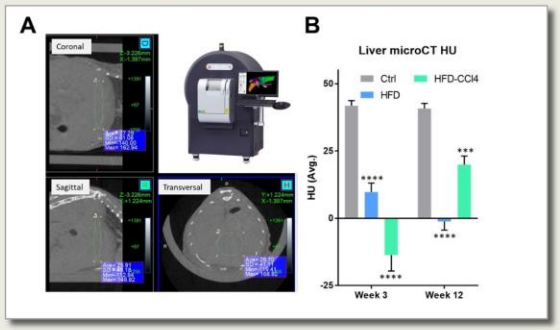
Fluorescence imaging of liver inflammation during the initial stage of NASH. Two weeks after NASH development, mice received i.v. injections of a fluorescent agent cocktail of IVISense Annexin-V 750, IVISense MMP 750, IVISense Transferrin Receptor 750 (AMT750, PerkinElmer Inc.). This cocktail can detect cell death, inflammation, and iron metabolism changes in the body. Another imaging probe, IVISense Vascular 680 (IV680, PerkinElmer Inc.), was also used to imaging vascular leakiness in inflamed tissues. The day after probe injection, liver fluorescent signals were measured using the (**A**) **IVIS® Spectrum** (PerkinElmer Inc.) and (**B**) **FMT® 4000** system (PerkinElmer Inc.). (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, t-test compared with Ctrl)

3 Longitudinal ultrasound imaging of NASH disease progression



(**A**) Robotic ultrasound **(Vega, SonoVol Inc.)** was used to perform SWE imaging on NASH mice. (**B**) In addition to traditional B-mode imaging, this automatic ultrasound system can perform shear wave elastography (SWE) to detect changes in liver stiffness. When performing SWE, a B-mode image is also acquired for overlay. In this example, a bright B-mode spot was identified which also showed high SWE stiffness reading (red arrow). (**C**) Longitudinal SWE and B-mode imaging of NASH livers. Lower liver stiffness was observed in the HFD-CCL4 livers three weeks after CCl4 induction, indicating fat accumulation and steatosis. However, after the 8th week, the HFD-CCl4 livers became stiffer than Ctrl (****P<0.0001, two-way ANOVA), suggesting extensive tissue fibrosis. B-mode imaging of the livers showed gradual increase of grayscale intensity as fat accumulated during NASH development. Although the HFD livers show no statistically significant change in SWE stiffness readings, they indeed had slightly higher B-mode grayscale intensities (***P<0.001, two-way ANOVA) when compared with the Ctrl livers. This observation is consistent with the low-level of fat deposits (microvesicular steatosis) seen in the ballooning hepatocytes of HFD livers.

4 MicroCT imaging of liver density at the early and late stage of NASH development



X-ray microCT imaging (**Quantum GX2**, PerkinElmer Inc.) was also performed to measure changes in liver tissue electron density due to fat accumulation and steatosis. (**A**) A typical liver microCT scanning showing the coronal, sagittal and transversal views of the liver. In each view, a representative area (green dash oval lines) was used to determine the average HU reading for each subject. (**B**) The microCT HU assessment of liver density in the NASH mice supports the SWE findings. Liver HU readings at week 3 of NASH development showed lower HU readings in the HFD and HFD-CCl4 livers, suggesting fat accumulation and steatosis. However, at week 12, the HFD-CCl4 livers showed recovery in HU, suggesting development of denser fibrosis in tissues. (***P<0.001, ****P<0.0001, t-test compared with Ctrl)

Conclusion

This study demonstrates that a multimodal *in vivo* imaging approach can be used to non-invasively detect, visualize, and quantify disease progression in animal NASH models. Three imaging modalities were tested, and each has its unique strength for detecting certain aspects of NASH. **(1) Fluorescence (FLI)** imaging uses highly specific molecular imaging probes for tissue inflammation, making early detection of liver inflammation possible. **(2) Ultrasound** imaging can visualize, detect and quantify subtle texture changes in soft tissue without the need of contrast agent or probe. In this NASH model, the shear wave elastography (SWE) mode can detect early reduction of tissue stiffness due to fat accumulation and steatosis. Interestingly, as the livers exposed to the chronic inflammatory condition induced by CCl4, SWE imaging indeed captures the gradual increase in liver stiffness due to fibrosis or possible cirrhosis at the later stage. The B-mode can be used to assess fat accumulation in the liver, as a higher fat content results in a higher grayscale intensity reading. **(3) MicroCT** can also measure changes in liver texture by assess the electron density. However, since it requires the use of ionizing radiation, microCT is not suitable for repeated measurements.

This multimodal strategy provides a novel approach for the non-invasive visualization and quantitative measurement of NASH disease progression. As each technology aims to visualize different aspects of the disease, this multimodal imaging strategy should facilitate the *in vivo* assessment of potential NASH treatments in preclinical models.