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Title: Application of Ultrasound and Shear Wave Elastography Imaging in a Rat Model of NAFLD/NASH

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Screen capture is not possible, videographer will film the screen**

   Videographer: *Film the screen for all* ***SCREEN*** *shots. The authors are not able to obtain screen capture videos.*

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19
Number of Shots: 39

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Dinesh Hirenallur-Shanthappa:** Use of ultrasound and shear wave elastography enables researchers to measure the changes in liver fat and fibrosis in preclinical models of nonalcoholic steatohepatitis.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.2. **Dinesh Hirenallur-Shanthappa:** This imaging modality is a non-invasive, high throughput, and a clinically translatable technique and can be used to assess the disease stage and efficacy in nonalcoholic steatohepatitis models during drug discovery.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-Roll: 4.4.2*

Introduction of Demonstrator on Camera

1.3. **Dinesh Hirenallur Shanthappa:** Demonstrating the procedure will be Jeffrey Morin, a Senior Scientist from my laboratory.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator looks up from the workbench or desk, or microscope and acknowledges the camera.

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Pfizer Inc.

Protocol

2. Preparation of Rat for Imaging and Instrument Set-up

2.1. To begin, place 6 to 7-week-old male Wister Han rats weighing 150 to 175 grams in pairs in individually ventilated cages with paper bedding **[1]** at approximately 22 degrees Celsius and 40 to 70% relative humidity with a 12 to 12-hour light-dark cycle **[2]**.

2.1.1. WIDE: Talent placing the rats in pairs in cages.

2.1.2. Temperature and humidity in the display panel of the cages. NOTE: Temperature could not be accessed.

2.2. Provide 20 rats with the choline-deficient, high-fat diet with 1% cholesterol **[1]** and another 20 rats with the standard lab rodent chow **[2].**

2.2.1. Talent feeding rats from the container labeled as a high-fat diet.

2.2.2. Talent feeding rats from the container labeled as standard lab rodent chow.

2.3. To set up the imaging instrument, place a warmed surface in the imaging area to keep the animal warm during imaging **[1]** and fix the anesthesia nose cone for delivering the inhalant anesthesia **[2]**.

2.3.1. Talent placing warmed surface in the imaging area.

2.3.2. Talent fixing the anesthesia nose cone in the imaging area.

2.4. Use the ultrasound probe holder to move the ultrasound probe to the desired location and to prevent the probe from resting on the animal **[1]**.

2.4.1. Talent moving the ultrasound probe using the probe holder.

2.5. Once the rat is completely anesthetized, transfer it from the induction chamber to a warm hot water circulating blanket **[1]**. Use chemical depilation cream to remove the hair from the ribcage to the pelvis on the right side of the rat **[2]**.

2.5.1. Talent transferring the rat from chamber to hot water circulating blanket.

2.5.2. Talent shaving the hair. NOTE: Two shots of shaving: one just with clippers and second with clippers and depilatory cream. *Video Editor: Please use the shot with clippers and depilatory cream*

2.6. Place the rat in the left lateral recumbent position and tape the upper paws above the head on a warm imaging platform **[1]**. Press the **Patient** key on the instrument control panel and identify the subject according to the study design **[2]**.

2.6.1. Talent placing the rat on the imaging platform and fixing paws with tape.

2.6.2. Talent pressing the patient key on the instrument control panel.

3. Image Acquisition for Hepato-Renal (HR) Index Measurement

3.1. Apply a small amount of warmed ultrasound gel on the depilated skin region of the rat **[1]**. Move the ultrasound probe to touch the gel-covered area **[2]**.

3.1.1. Talent applying gel on the shaved area of the rat.

3.1.2. Talent placing the ultrasound probe on the gel-covered area.

3.2. Once the live B-mode image of the internal organs appears on the screen **[1]**, move the ultrasound probe to the area slightly above the hip, just parallel to the lumbar vertebrae **[2]**.

3.2.1. SCREEN: The B-mode image of the internal organs appearing on the screen. ***Videographer:*** *Film the screen for all* ***SCREEN*** *shots. The authors are not able to obtain screen capture videos.*

3.2.2. Talent moving the ultrasound probe to the area slightly above the hip.

3.3. Use B-mode display to locate the right kidney by identifying the large renal artery and cortex-medulla separation. Observe a part of the liver in a single plane of the image and ensure that air bubbles or shadows are not present in the image **[1]**.

3.3.1. SCREEN: Right kidney located and liver indicated with the cursor in the image.

3.4. Adjust the focus **[1]** and make sure that the renal cortex and liver parenchyma are in the same plane to obtain a clear image **[2]**. Ensure that the animal is between breaths when freezing the screen to avoid capturing blurry images **[3]**.

3.4.1. Talent turning the focus knob on the control panel.

3.4.2. SCREEN: Focus adjusted, renal cortex and the liver parenchyma indicated by cursor in the same plane.

3.4.3. Talent pressing the freeze key on the control panel.

3.5. Select the **B-mode Ratio** to measure the relative brightness of tissue from the selected region of interest **[1]**. Create a 2-millimeter circle and place it on the region of interest in the image of the liver, which is located to the right of the kidney **[2]**. *Videographer: This step is important!*

3.5.1. SCREEN: B-mode ratio selected.

3.5.2. SCREEN: Circle created and placed on the region of interest.

3.6. Place a new circle on the image of the kidney cortex, keeping the depths of the circles on the liver and kidney cortex the same **[1]**. *Videographer: This step is important!*

3.6.1. SCREEN: Circle placed on the kidney cortex image and depth of both circles shown.

3.7. Press the **Select** button on the control panel **[1]** to display the hepato-renal index as a B-mode ratio and repeat the measurement 3 times at different depths and planes of the tissue, then calculate the average **[2]**. *Videographer: This step is important!*

3.7.1. Talent pressing select button on the control panel.

3.7.2. SCREEN: B-mode ratio displayed.

4. Image Acquisition for the Shear Wave Elastography

4.1. Move the probe transversely in the right subcostal area to locate the liver using B Mode **[1]**. Locate a clear area of the liver that is mostly parenchyma and free of large blood vessels such as the portal vein and hepatic artery **[2]**. *Videographer: This step is important!*

4.1.1. Talent moving the probe transversely in the right subcostal area.

4.1.2. SCREEN: Clear area of liver located.

4.2. Then, press the **SWE** button on the control panel **[1]** to generate a shear elasticity map of the tissue **[2]**. Adjust the size and position of the SWE box below the liver capsule in an area that is free of shadows **[3]**. When the box is full and stable, press the **Freeze** button on the control panel while the rat is between breaths **[4]**. *Videographer: This step is important!*

4.2.1. Talent pressing the swe button on the control panel.

4.2.2. SCREEN: Shear elasticity map generated.

4.2.3. SCREEN: Size of the SWE box adjusted and box placed below the liver capsule.

4.2.4. Talent pressing the freeze button on the control panel.

4.3. Tap the **QBox** on the touch display of the instrument to compute the elasticity from the region of interest on the shear wave elasticity map **[1]**. A circle and data box will appear. Position the circle in the shadow-free area with uniform coloring, avoiding areas of stiffness **[2]**. *Videographer: This step is important!*

4.3.1. Talent tapping the qbox on the touch display.

4.3.2. SCREEN: Circle and data box appearing, and circle positioned.

4.4. Repeat the procedure 3 times by moving the probe up and down or sideways on the abdomen to gather SWE-mapped images from different areas of the liver **[1]**. When finished, remove the tape from the paws **[2]** and wipe the excess gel **[3].** Place the rat back in a warm and dry cage and allow it to recover fully **[4]**.

4.4.1. Talent moving the probe up and down on the abdomen area.

4.4.2. Talent removing the tape from paws.

4.4.3. Talent wiping the excess gel.

4.4.4. Talent placing the rat back in a warm and dry cage.

5. Image Data Retrieval

5.1. Select all scans required for data analysis by checking the box next to the patient's name using the trackball and **Select** button **[1]**.

5.1.1. Talent rolling the trackball and pressing the select button.

5.2. After exporting the files, open an individual jpg *(J-P-G)* file of each scan and observe the data on the right side of the image **[1]**. Copy all data into a spreadsheet or other database management software and perform the desired statistical analyses **[2]**.

5.2.1. SCREEN: JPG file opened, and data on the right side highlighted with a cursor.

5.2.2. SCREEN: Data copied to a spreadsheet. NOTE: This shot was not filmed

Results

6. Results: Analysis of the Hepato-renal Index and Shear Wave Elastography in High-fat and Control Diet Groups

6.1. The representative images of hepato-renal indices from the control and choline-deficient, high-fat diet groups **[1]** show that the brightness of the liver and the renal cortex was almost the same and the hepato-renal index was less than 1 in the control group **[2]**.

6.1.1. LAB MEDIA: Figure 4

6.1.2. LAB MEDIA: Figure 4A *Video Editor: Please emphasize the B Ratio in the 6- and 12-week control images.*

6.2. In the high-fat diet group, the brightness of the liver increased, and the hepato-renal index was elevated up to 1.91 at 6-weeks **[1]** and 1.79 at 12-week time points **[2]**.

6.2.1. LAB MEDIA: Figure 4A *Video Editor: Please emphasize the B Ratio in the 6-week CDAHFD image.*

6.2.2. LAB MEDIA: Figure 4A *Video Editor: Please emphasize the B Ratio in the 12-week CDAHFD image.*

6.3. The hepato-renal index values of the high-fat diet group rose quickly throughout the first 3 to 6 weeks **[1]** before reaching a plateau **[2]**.

6.3.1. LAB MEDIA: Figure 4C *Video Editor: Please emphasize the red line moving upward between 3- and 6-week time points in the graph.*

6.3.2. LAB MEDIA: Figure 4C *Video Editor: Please emphasize the red line moving downward and then straight between 6- and 12-week time points in the graph.*

6.4. Liver sections stained with oil red-o dye showed **[1]** a significantly increased stained area in the high-fat diet group **[2]** compared to the control group **[3]**. A correlation was also found between the %-stained area and the hepato-renal index **[4]**.

6.4.1. LAB MEDIA: Figure 4B and D

6.4.2. LAB MEDIA: Figure 4B and D *Video Editor: Please emphasize 6- and 12-week CHAHFD images in figure 4B and the area with the red squares in the graph in figure 4D.*

6.4.3. LAB MEDIA: Figure 4B and D *Video Editor: Please emphasize 6- and 12-week control images in figure 4B and the area with the blue circles near the baseline in the graph in figure 4D.*

6.4.4. LAB MEDIA: Figure 4E

6.5. Tissue stiffness **[1]** increased gradually in the high-fat diet group over 12 weeks and reached 23.1 kilopascals due to fibrosis **[2]**.

6.5.1. LAB MEDIA: Figure 5C

6.5.2. LAB MEDIA: Figure 5C *Video Editor: Please emphasize the red line in the graph.*

6.6. The liver samples were stained with picro-sirius red to localize collagen as an indicator of fibrosis **[1]**. A significant increase of the %-stained area was observed in the high-fat diet group **[2]** compared to the control group **[3]**.

6.6.1. LAB MEDIA: Figure 5D

6.6.2. LAB MEDIA: Figure 5D *Video Editor: Please emphasize the area with red squares in the graph.*

6.6.3. LAB MEDIA: Figure 5D *Video Editor: Please emphasize the area with blue circles in the graph.*

6.7. A strong correlation was also found between the % -stained area and the shear wave E-modulus numbers **[1]**.

6.7.1. LAB MEDIA: Figure 5E

Conclusion

7. Conclusion Interview Statements

7.1. **Terri Swanson:** It is important to place the transducer and measurement circles or boxes properly. Avoiding artifacts in the images will allow for repeatable and consistent measurements.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-Roll: 3.1.2 and 4.2.3*

7.2. **Jeff Morin :** After this procedure, *ex-vivo* measurements of liver triglycerides and collagen gene expression can also be performed on necropsied tissue to confirm the results seen in the imaging.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera