

## Appendix E1

### MR Elastography–assessed Mechanical Properties and Rationale of Parameter Selection

The value of independent mechanical properties other than shear stiffness in distinguishing different pathophysiologic statuses of the liver remains to be established. These quantities include model-free properties (eg, the complex shear modulus, longitudinal strain, and frequency dispersion of mechanical properties) and model-based viscoelastic parameters (8,19,37–44). Generally, tissue mechanical properties, such as shear wave speed, attenuation, storage modulus, and loss modulus, will increase with frequency and can be modeled by a complex wave number (45). Whatever the exact mechanisms of loss are in a given medium, this dispersion of the mechanical properties is governed in part by Kramers-Kronig relations that are based on causality constraints on the tissue motion (46). With the development of elastographic imaging approaches over the past two decades (47), some techniques have been extended to examine dispersion in healthy and diseased tissues, including breast cancer (48,49), liver fibrosis (9,38,50), muscle (51–53), and healthy mammalian livers (54,55), as well as in gelatins (56). Reported quantities include velocity dispersion, attenuation dispersion, storage modulus dispersion, loss modulus dispersion, and stiffness (magnitude of complex shear modulus) dispersion. Some of the magnetic resonance (MR) elastography inversion techniques currently used (eg, direct inversion) allow for basic viscoelastic modeling of tissue, meaning that energy-losing mechanisms (eg, viscosity and attenuation) can be accounted for in addition to the elastic properties of the tissue. This information is typically expressed via a complex-valued shear modulus  $G^* = G' + iG''$  in the equations of motion (38,57). Several mechanical properties can be immediately derived from the direct inversion calculation, including storage modulus  $\text{Re}(G^*)$  or  $G'$ , loss modulus  $\text{Im}(G^*)$  or  $G''$ , shear stiffness  $|G^*|$ , damping ratio  $\zeta = G''/(2G')$ , wave speed, and attenuation, some of which may improve the diagnostic capabilities of elastography when used alone or in combination. Different viscoelastic models have been proposed to explain this frequency dependence in soft tissues or to characterize it with a small number of parameters (8,19,37–40). However, we did not apply viscoelastic models in this work in consideration of the difference in disease origins and the possible bias observed (8,19,37–40).

### Animal Models

Animal models of five different liver diseases were studied to represent a variety of conditions: knockout autosomal recessive polycystic kidney disease (ARPKD), carbon tetrachloride ( $\text{CCl}_4$ )-induced liver disease, nonalcoholic fatty liver disease (NAFLD), hepatic venous congestion, and fumarylacetoacetate hydrolase (FAH)-deficient diseases. Each model had its own detailed protocol for imaging and histologic analysis, as will be described.

### ARPKD Mouse Model

Hepatic inflammation and fibrosis were demonstrated in a knockout ARPKD mouse model (58) with congenital chronic liver injury: A total of 18 mice with ARPKD and 18 age- and sex-matched control mice aged 1, 3, and 6 months were studied. They were all sacrificed

immediately after MR elastography data collection at scheduled times for tissue harvesting and histologic analysis.

### **CCl<sub>4</sub> Mouse Model**

Hepatic inflammation and fibrosis were demonstrated in C57BL/6 wild-type mice (Jackson Laboratories, Bar Harbor, Me) with prolonged intraperitoneal administration of 1  $\mu$ L per gram of body weight CCl<sub>4</sub> mixed with olive oil (experimental group) or olive oil alone (control group) twice a week: A total of 12 CCl<sub>4</sub> and 12 age-matched control male mice underwent imaging 1, 2, 4, and 6 weeks after initiation of injection. They were all sacrificed immediately after MR elastography data collection at scheduled times for tissue harvesting and histologic analysis.

### **NASH Mouse Model**

Hepatic steatosis, inflammation, and fibrosis were demonstrated in an NAFLD mouse model, covering a wide range of nonalcoholic fatty liver (NAFL) or simple steatosis to nonalcoholic steatohepatitis (NASH) through the animal's life time: A total of 55 C57BL/6 wild-type male mice on a high-fat diet (59) with NAFL or NASH and 40 control male mice of the same age underwent monthly imaging (Fig E1). They were sacrificed for histologic analysis at 1, 12, 24, 36, or 48 weeks of feeding.

### **pIVCL Mouse Model**

Hepatic venous congestion was demonstrated in C57BL/6 wild-type mice with partial inferior vena cava ligation (pIVCL) surgery (14): A total of 16 mice with pIVCL and 11 control mice of the same age underwent imaging 2, 4, and 6 weeks after surgery. All mice were sacrificed immediately after MR elastography data collection at scheduled times for portal pressure measurement, then tissue harvesting and histologic analysis were performed.

### **FAH Pig Model**

Hepatic fibrosis and portal hypertension were demonstrated in an FAH-deficient pig model (60) and were maintained with a low dose of nitisinone: All five pigs had inborn FAH-deficient disease and underwent MR elastography monthly (Fig E1). Euthanasia was scheduled for 12, 24, or 36 months of age, unless the pigs showed poor vital signs.

## **Justifications of Animal Numbers and Data Distributions**

A priori power analysis was performed with our preliminary experience in mouse liver MR elastography. Assuming mean stiffness of healthy liver tissues is around 1.0 kPa  $\pm$  0.1 (standard deviation) at 200 Hz and that MR elastography is expected to depict a 20% increase in stiffness (0.2 kPa), we can achieve 90% power with a two-tailed matched *t* test with a total sample size of six animals (the effect size is 1.796). Thus, we determined a sample size of three experimental and three control mice in our matched comparisons was adequate for the ARPKD and CCl<sub>4</sub> mouse models.

In the NAFLD mouse models, we did not use the matched strategy. To detect the same 20% stiffness increase between two independent groups, we need a total sample size of 12 animals (at least six mice for each subgroup) to achieve at least 80% power.

In the pIVCL model, no detectable fibrosis developed before 6 weeks after surgery, and only the portal pressure would affect the mechanical properties of the liver (61). If we assume a linear regression with fixed model and single regression coefficient, we can achieve 96% power with a total sample size of 16 in a two-tailed test to detect a substantial correlation with coefficient of determination greater than 0.5.

Use of the NAFLD models is expensive and time consuming. We did not want to risk starting it over if any unexpected big loss occurred during the early phase of gaining experience (ie, first data set of week 1) and the later phase of severe liver diseases (ie, weeks 36–48). Thus, we ordered extra mice to compensate for possible large animal losses in those two phases. Additionally, it has been well established that the more severe hepatic fibrosis is, the more heterogeneous the liver tissue will be. That is to say, animals in the experimental group should have slightly larger variance than that in control animals. Thus, we have different numbers of mice in each subgroup.

We applied Kolmogorov-Smirnov tests for each animal model to validate data distribution of multiple parameters ( $G'$ ,  $G''$ ,  $|G^*|$ , and  $\zeta$ ) in all our comparisons and regression analyses. According to the normality validation, we applied the two-tailed Welch  $t$  test for all mean value comparisons in ARPKD, CCl<sub>4</sub>, and NASH mouse models and the Spearman correlation for the pIVCL mouse model.

## MR Elastography Preparation

Mice were anesthetized with 1.0%–1.5% isoflurane according to their body weight. After preparation of the abdomen and administration of maintenance anesthesia with isoflurane, each mouse was placed in a plastic cradle in the supine position, then slid into a custom eight-channel birdcage imaging coil with a 4-cm inner diameter. A disposable silver acupuncture needle with a 0.40-mm diameter and 39-mm length (Asahi Medical Instrument, Kawaguchi, Saitama, Japan) was inserted into the liver tissue through the anterior body wall. The other end of the needle was connected to an electromechanical driver, which generated longitudinally oriented sinusoidal vibrations at seven different frequencies from 80 to 200 Hz with an increment of 20 Hz, producing a cylindrically symmetric shear wave field within the liver tissue (17). Pigs were sedated with a cocktail mix of telazol, xylazine, and glycopyrolate. An endotracheal tube was placed for ventilation and administration of maintenance anesthesia with isoflurane during imaging. A four-channel phased-array surface coil was used for imaging. The pig was imaged in the supine position with two acoustic pressure-activated drivers placed over the right and left side of the body wall to generate continuous sinusoidal vibrations at 60, 80, and 100 Hz throughout the abdomen (24).

## MR Elastography Imaging

After MR elastography preparation, imaging was performed with a 3.0-T (mice) or 1.5-T (pigs) whole-body imager (HDx; GE Healthcare, Milwaukee, Wis). MR elastography wave images at multiple frequencies of mechanical vibration were acquired with a free-breathing four-shot (mice) or suspended-breathing two-shot (pigs) multisection spin-echo echo planar MR elastography sequence using three alternating orthogonal motion-encoding directions with a  $96 \times 96$  in-plane acquisition matrix (repetition time msec/echo time msec, 400/37.5–43.5 in mice, 3000/47–53 in pigs; section thickness, 2 mm in mice and 5 mm in pigs; and four evenly spaced phase offsets over one motion cycle). The encoded motion sensitivities were 13–25  $\mu\text{m per } \pi$

radians for frequencies of 80–200 Hz in mice and 37, 20, and 15  $\mu\text{m}$  per  $\pi$  radians for 60-, 80-, and 100-Hz motion in pigs, respectively. The field of view was 8–38 cm, and the number of sections was between eight and 42 depending on the size of the animals. The acquisition time was 2–3 minutes in mice and 4–5 minutes in pigs for each individual frequency.

## MR Elastography Image Processing and Calculation of Mechanical Properties

All MR elastography wave data were interpolated in-plane to  $256 \times 256$  (matrix preparation for postprocessing with fixed parameters of kernel size and filters) and were (a) processed with the curl operator (using central differences), (b) processed with 20 evenly spaced three-dimensional directional filters (61) (radial fourth-order Butterworth bandpass filter, cutoff frequencies of 0.001 and 24 cycles per field of view), (c) smoothed with a  $3 \times 3 \times 3$  quartic kernel (62), and (d) inverted with direct inversion of the Helmholtz equation (63) to calculate the complex shear modulus  $G^* = G' + iG''$  at each frequency. Several mechanical properties were derived from  $G^*$ , including storage modulus ( $G'$ ), loss modulus ( $G''$ ), shear stiffness ( $|G^*|$ ), and damping ratio ( $\zeta$ ). Note that the damping ratio ( $\zeta = G''/(2G')$ ) carries the same information as the loss tangent ( $G''/G'$ ), the quality factor ( $G'/G''$ ), and the phase angle ( $\tan^{-1}[G''/G']$ ) and that all these quantities are easily converted to each other. For each animal at each time point, all quantities ( $G'$ ,  $G''$ ,  $G^*$ ,  $\zeta$ ) were reported as a one-time volumetric measurement set of means and standard deviations (ie, intraregion of interest variability) of regions of interest manually drawn to encompass as much of the liver as possible that had substantial wave propagation at visual evaluation (M.Y., >10 years of experience in liver MR elastography). The criteria for region of interest placement were as follows: (a) include liver parenchyma only, (b) exclude regions without visually adequate magnitude signal or shear wave amplitude, (c) exclude the location of the vibrating needle and the adjacent area (circular area with a three-pixel radius) in mice, and (d) keep two pixels away from the edges and exclude the top and bottom two sections of the liver. Finally, the means and standard deviations of four different mechanical parameters ( $G'$ ,  $G''$ ,  $G^*$ ,  $\zeta$ ) were reported for each subgroup of animals at each time point.

## Histologic Analysis, Blood Test, and Portal Pressure Measurement

Histologic analysis was performed with hematoxylin-eosin (64) and picosirius red staining (65) of formalin-fixed paraffin-embedded 5- $\mu\text{m}$  liver slices in mice or percutaneous liver biopsy samples in pigs (obtained every 3 months). The histologic features were assessed with the NASH clinical research network scoring system (66) and the Ishak scoring system (67), when appropriate (T.M., 6 years of experience in liver histology). The NAFLD activity score (NAS) (66), which is defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2), was also used in the NASH mouse model because of the consistently observed histologic feature of patterns and lesions (68). In the pIVCL mouse model, fibrosis extent was determined by using the hydroxyproline content in the whole liver specimen, which was quantified colorimetrically, as described by Yang et al (69). Hydroxyproline concentration was calculated from a standard curve prepared with high-purity hydroxyproline (Sigma) and expressed as micrograms per milligram of liver tissue. Histologic analysis was performed by authors (T.M., 6 years of experience in liver pathology for NASH, ARPKD, CCl<sub>4</sub> groups; D.A.S., 1 year of experience in liver pathology for pIVCL; J.M.G., 1 year

of experience in liver pathology for FAH) who were blinded to MR results at the time of independent evaluation in all cases.

Alanine aminotransferase (ALT) tests were performed (H.M.) in the NAFLD mouse model via cardiac puncture to evaluate liver function.

Portal pressure was measured in the pIVCL mice (D.A.S.) and in the pigs (J.M.G.) immediately prior to sacrifice. In mice, it was directly measured by using a digital blood pressure analyzer (Digi-Med; Micro-Med, Louisville, Ken) with a computer interface (70). Once the analyzer was calibrated, a 16-gauge catheter attached to the pressure transducer was inserted into the portal vein and sutured in place. The pressure was continuously monitored, and the average portal pressure was recorded. In pigs, it was measured via portal and hepatic vein pressures to determine a final portocaval gradient.

## **Histologic Images and Analysis of ARPKD Mouse Model**

Figure E2 shows eight microscopic images, including healthy hepatic parenchyma, from the control groups and diseased liver tissue with progressively increasing fibrosis extent from F1 to F4 (Ishak scoring system, F0–F6) and inflammation grade from I0 to I2 (Ishak scoring system, I0–I4).

## **Histologic Analysis of NASH Mouse Model**

In histologic analyses of 14 different features suggested by Kleiner et al (66), we selected seven histologic features and ALT level to show in Figure E2c–E2e. The location of predominant fat distribution pattern was zone 3 in control animals, zone 1 or Azona in the livers of animals with NAFL before week 12, and paracinar in borderline or definite NASH livers after week 12. Other histologic features (six of 14) did not show significant changes across different age groups of mice with NAFLD or when we compared each age group with its matched control group, as shown in Table E2. Steatosis extent reached its peak at week 24 in the histologic assessment of Figure E2c and so did fat fraction at week 12 in the MR imaging assessment of Figure E2f. The body weight measurement of Figure E2f increased consistently in this mouse model. Both histologic steatosis grade and MR imaging–assessed fat fraction measurements remained high afterward. In the hepatic inflammation model, lobular inflammation decreased at week 12, then increased at week 24 and afterward; microgranulomas were present at week 1, absent from week 12 to week 24, and increased at week 48; portal inflammation was evident at only week 48. Abnormal presence of or increased steatosis and inflammation was seen as early as week 1. For hepatocellular injury, ballooning started to manifest at week 24 and increased in severity thereafter. Pigmented macrophages, megamitochondria, and Mallory hyaline showed their presence only at week 48. There was no or minimal hepatic fibrosis before week 12, mild fibrosis at week 24, and significant to severe fibrosis increasingly developed from week 36 to week 48. Hepatic fibrosis and hepatocellular ballooning were not histologically detected as stage or grade 1 until week 24. There was minimal necroinflammation, and no fibrosis was observed in the livers of age-matched control animals. The mean NAFLD activity scores in mice with NASH were  $1.8 \pm 0.4$ ,  $3.1 \pm 1.3$ ,  $5.1 \pm 1.3$ , and  $6.8 \pm 1.0$  at weeks 1, 12, 24, and 48, respectively.

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**Table E1: Welch T Test Results of Multifrequency Mechanical Properties in the NAFLD, NASH, Knockout ARPKD, and CCl<sub>4</sub> Mouse Models**

A: ARPKD Mouse Model with Congenital Chronic Liver Disease (No. of Animals Studied: ARPKD, n = 18; Control, n = 18)																
Freq (Hz)	Storage Modulus			Loss Modulus			Shear Stiffness			Damping Ratio						
	1 Mo	3 Mo	6 Mo	1 Mo	3 Mo	6 Mo	1 Mo	3 Mo	6 Mo	1 Mo	3 Mo	6 Mo				
80																
Disease (n = 6)	0.27 ± 0.03	0.39 ± 0.03	0.43 ± 0.06	0.028 ± 0.006	0.013 ± 0.009	0.008 ± 0.006	0.28 ± 0.03	0.39 ± 0.02	0.43 ± 0.07	0.055 ± 0.005	0.017 ± 0.007	0.009 ± 0.004				
Control (n = 6)	0.24 ± 0.02	0.28 ± 0.02	0.27 ± 0.03	0.006 ± 0.004	0.012 ± 0.007	0.008 ± 0.006	0.25 ± 0.02	0.28 ± 0.02	0.27 ± 0.03	0.012 ± 0.004	0.022 ± 0.008	0.013 ± 0.007				
P value	.02*	<.001*	<.01*	<.001*	.85	.92	.01*†	<.001*†	<.001*†	<.001*†	.53	.53				
100																
Disease (n = 6)	0.36 ± 0.06	0.52 ± 0.05	0.65 ± 0.09	0.058 ± 0.033	0.018 ± 0.008	0.039 ± 0.034	0.37 ± 0.06	0.52 ± 0.05	0.66 ± 0.08	0.075 ± 0.021	0.017 ± 0.004	0.032 ± 0.018				
Control (n = 6)	0.35 ± 0.05	0.42 ± 0.02	0.39 ± 0.04	0.022 ± 0.009	0.014 ± 0.011	0.019 ± 0.010	0.35 ± 0.05	0.42 ± 0.02	0.39 ± 0.04	0.031 ± 0.006	0.017 ± 0.008	0.024 ± 0.009				
P value	.55	<.01*	<.001*	.04*	.51	.26	.63	<.01*	<.001*	.03*	.99	.63				
160																
Disease (n = 6)	0.69 ± 0.07	0.91 ± 0.11	1.14 ± 0.15	0.060 ± 0.027	0.031 ± 0.018	0.106 ± 0.058	0.70 ± 0.08	0.91 ± 0.12	1.15 ± 0.15	0.044 ± 0.012	0.015 ± 0.007	0.045 ± 0.014				
Control (n = 6)	0.60 ± 0.10	0.76 ± 0.06	0.73 ± 0.09	0.074 ± 0.062	0.076 ± 0.045	0.061 ± 0.039	0.61 ± 0.12	0.77 ± 0.06	0.74 ± 0.09	0.054 ± 0.025	0.050 ± 0.018	0.039 ± 0.015				
P value	.21	.06	<.001*	.69	.08	.22	.18	.04*	<.001*	.99	.13	.73				
200																
Disease (n = 6)	1.06 ± 0.19	1.20 ± 0.07	1.65 ± 0.07	0.094 ± 0.096	0.038 ± 0.019	0.139 ± 0.089	1.07 ± 0.21	1.20 ± 0.07	1.65 ± 0.30	0.037 ± 0.019	0.016 ± 0.004	0.041 ± 0.012				
Control (n = 6)	0.97 ± 0.11	1.08 ± 0.11	0.98 ± 0.15	0.086 ± 0.019	0.043 ± 0.028	0.032 ± 0.026	0.97 ± 0.13	1.08 ± 0.11	0.98 ± 0.15	0.045 ± 0.007	0.020 ± 0.009	0.017 ± 0.009				
P value	.43	.05	<.01*	.87	.73	.06	.43	.04*	<.01*	.40	.54	.09				
B: CCl <sub>4</sub> Mouse Model with Drug-induced Chronic Liver Disease (No. of Animals Studied: CCl <sub>4</sub> , n = 12; Control, n = 12)																
Freq (Hz)	Storage Modulus				Loss Modulus				Shear Stiffness				Damping Ratio			
	1 Wk	2 Wk	4 Wk	6 Wk	1 Wk	2 Wk	4 Wk	6 Wk	1 Wk	2 Wk	4 Wk	6 Wk	1 Wk	2 Wk	4 Wk	6 Wk
80																
Disease (n = 3)	0.32 ± 0.03	0.27 ± 0.03	0.31 ± 0.04	0.31 ± 0.01	0.011 ± 0.009	0.010 ± 0.007	0.013 ± 0.004	0.010 ± 0.006	0.34 ± 0.03	0.28 ± 0.04	0.32 ± 0.04	0.32 ± 0.02	0.018 ± 0.017	0.020 ± 0.013	0.022 ± 0.008	0.015 ± 0.009
Control (n = 3)	0.29 ± 0.04	0.29 ± 0.03	0.29 ± 0.02	0.27 ± 0.04	0.015 ± 0.008	0.017 ± 0.010	0.012 ± 0.005	0.012 ± 0.006	0.30 ± 0.05	0.31 ± 0.04	0.31 ± 0.02	0.28 ± 0.04	0.027 ± 0.014	0.029 ± 0.015	0.021 ± 0.007	0.022 ± 0.012
P value	.22	.44	.48	.16	.43	.27	.78	.64	.23	.39	.50	.14	.43	.19	.62	.73
100																

Disease (n= 3)	0.46 ± 0.03	0.42 ± 0.03	0.44 ± 0.02	0.47 ± 0.01	0.019 ± 0.012	0.017 ± 0.010	0.018 ± 0.019	0.018 ± 0.009	0.49 ± 0.04	0.43 ± 0.04	0.46 ± 0.03	0.49 ± 0.01	0.022 ± 0.015	0.021 ± 0.012	0.020 ± 0.020	0.019 ± 0.010
Control (n= 3)	0.42 ± 0.07	0.44 ± 0.07	0.44 ± 0.04	0.40 ± 0.04	0.025 ± 0.013	0.029 ± 0.009	0.011 ± 0.008	0.026 ± 0.013	0.44 ± 0.07	0.46 ± 0.08	0.46 ± 0.04	0.41 ± 0.05	0.032 ± 0.020	0.032 ± 0.020	0.011 ± 0.008	0.033 ± 0.017
P value	.34	.65	.95	.01*	.52	.12	.42	.34	.32	.58	.99	.01*	.60	.15	.28	.56
160																
Disease (n= 3)	0.80 ± 0.13	0.76 ± 0.06	0.82 ± 0.07	0.85 ± 0.07	0.078 ± 0.016	0.045 ± 0.025	0.062 ± 0.035	0.049 ± 0.017	0.84 ± 0.14	0.79 ± 0.06	0.86 ± 0.07	0.88 ± 0.07	0.049 ± 0.014	0.029 ± 0.016	0.038 ± 0.022	0.029 ± 0.011
Control (n= 3)	0.72 ± 0.08	0.80 ± 0.08	0.74 ± 0.04	0.75 ± 0.04	0.071 ± 0.037	0.089 ± 0.037	0.052 ± 0.031	0.046 ± 0.030	0.75 ± 0.08	0.83 ± 0.09	0.77 ± 0.04	0.77 ± 0.04	0.049 ± 0.027	0.049 ± 0.027	0.035 ± 0.021	0.031 ± 0.022
P value	.20	.54	.07	.02*	.75	.07	.68	.85	.20	.48	.06	.02*	.92	.10	.73	.89
200																
Disease (n= 3)	1.07 ± 0.16	1.04 ± 0.08	1.11 ± 0.04	1.14 ± 0.02	0.134 ± 0.038	0.062 ± 0.030	0.072 ± 0.033	0.084 ± 0.055	1.11 ± 0.18	1.08 ± 0.09	1.16 ± 0.03	1.18 ± 0.02	0.062 ± 0.013	0.030 ± 0.015	0.032 ± 0.014	0.037 ± 0.025
Control (n= 3)	0.98 ± 0.09	1.07 ± 0.07	1.00 ± 0.06	1.03 ± 0.04	0.079 ± 0.029	0.106 ± 0.047	0.081 ± 0.022	0.079 ± 0.016	1.01 ± 0.10	1.11 ± 0.07	1.04 ± 0.06	1.06 ± 0.04	0.040 ± 0.013	0.040 ± 0.013	0.040 ± 0.012	0.038 ± 0.009
P value	.25	.66	.03*	<.01*	.01*	.13	.65	.85	.20 <sup>†</sup>	.63 <sup>†</sup>	.02 <sup>†</sup>	<.01* <sup>†</sup>	.02* <sup>†</sup>	.19 <sup>†</sup>	.41 <sup>†</sup>	.87 <sup>†</sup>
<b>C: NAFLD and NASH Mouse Models with Fast-Food Diet (No. of Studied Animals: NASH, n= 55;Control, n= 40)</b>																
Freq (Hz)	Storage Modulus				Loss Modulus				Shear Stiffness				Damping Ratio			
	8 Wk (8 M, 6 F)	12 Wk (8 M, 6 F)	20 Wk (8 M, 6 F)	48 Wk (10 M, 6 F)	8 Wk (8 M, 6 F)	12 Wk (8 M, 6 F)	20 Wk (8 M, 6 F)	48 Wk (10 M, 6 F)	8 Wk (8 M, 6 F)	12 Wk (8 M, 6 F)	20 Wk (8 M, 6 F)	48 Wk (10 M, 6 F)	8 Wk (8 M, 6 F)	12 Wk (8 M, 6 F)	20 Wk (8 M, 6 F)	48 Wk (10 M, 6 F)
80																
Disease	0.30 ± 0.30	0.28 ± 0.02	0.32 ± 0.04	0.47 ± 0.10	0.017 ± 0.010	0.018 ± 0.005	0.028 ± 0.010	0.031 ± 0.019	0.31 ± 0.03	0.30 ± 0.03	0.33 ± 0.04	0.51 ± 0.12	0.028 ± 0.017	0.031 ± 0.009	0.044 ± 0.012	0.031 ± 0.016
Control	0.29 ± 0.03	0.28 ± 0.02	0.29 ± 0.02	0.28 ± 0.02	0.017 ± 0.008	0.012 ± 0.008	0.008 ± 0.005	0.019 ± 0.005	0.30 ± 0.03	0.29 ± 0.02	0.31 ± 0.02	0.29 ± 0.01	0.028 ± 0.012	0.021 ± 0.013	0.014 ± 0.008	0.033 ± 0.007
P value	.74	.88	.32	.02*	.99	.17	<.01*	.35	.64 <sup>†</sup>	.79 <sup>†</sup>	.37 <sup>†</sup>	.02* <sup>†</sup>	.93 <sup>†</sup>	.093 <sup>†</sup>	<.001* <sup>†</sup>	.98 <sup>†</sup>
100																
Disease	0.44 ± 0.03	0.43 ± 0.04	0.44 ± 0.04	0.64 ± 0.12	0.026 ± 0.017	0.033 ± 0.015	0.037 ± 0.013	0.043 ± 0.021	0.46 ± 0.03	0.45 ± 0.04	0.46 ± 0.04	0.68 ± 0.13	0.030 ± 0.019	0.038 ± 0.015	0.041 ± 0.015	0.034 ± 0.016
Control	0.44 ± 0.03	0.41 ± 0.03	0.43 ± 0.02	0.42 ± 0.02	0.025 ± 0.016	0.017 ± 0.019	0.026 ± 0.012	0.043 ± 0.013	0.45 ± 0.04	0.43 ± 0.03	0.45 ± 0.02	0.45 ± 0.03	0.028 ± 0.016	0.020 ± 0.022	0.031 ± 0.014	0.050 ± 0.016
P value	.67	.42	.46	.02*	.88	.10	.10	.99	.62	.39	.64	.03*	.92	.11	.02*	.53

160																
Disease	0.74 ± 0.04	0.72 ± 0.03	0.71 ± 0.06	1.05 ± 0.17	0.073 ± 0.024	0.124 ± 0.027	0.112 ± 0.038	0.078 ± 0.016	0.78 ± 0.04	0.77 ± 0.03	0.75 ± 0.07	1.10 ± 0.17	0.048 ± 0.015	0.076 ± 0.015	0.079 ± 0.027	0.038 ± 0.011
Control	0.74 ± 0.04	0.74 ± 0.08	0.72 ± 0.03	0.75 ± 0.02	0.054 ± 0.023	0.085 ± 0.028	0.099 ± 0.016	0.120 ± 0.074	0.77 ± 0.04	0.78 ± 0.08	0.75 ± 0.03	0.80 ± 0.02	0.037 ± 0.016	0.048 ± 0.015	0.069 ± 0.011	0.078 ± 0.048
P value	.69	.34	.66	.02*	.17	.02*	.46	.25	.57	.46	.75	.03*	.15	.003*	.25	.12
200																
Disease	0.96 ± 0.05	0.92 ± 0.03	0.93 ± 0.03	1.25 ± 0.17	0.134 ± 0.036	0.150 ± 0.040	0.143 ± 0.041	0.158 ± 0.032	1.01 ± 0.06	0.98 ± 0.04	0.98 ± 0.03	1.14 ± 0.15	0.069 ± 0.017	0.081 ± 0.022	0.076 ± 0.023	0.064 ± 0.016
Control	1.01 ± 0.07	1.02 ± 0.10	1.01 ± 0.03	0.93 ± 0.04	0.087 ± 0.024	0.104 ± 0.016	0.163 ± 0.046	0.120 ± 0.040	1.05 ± 0.08	1.06 ± 0.11	1.06 ± 0.04	0.91 ± 0.03	0.043 ± 0.012	0.051 ± 0.009	0.081 ± 0.021	0.064 ± 0.021
P value	.15	.02*	<.01*	.02*	.01*	.02*	.39	.19	.33 <sup>†</sup>	.09 <sup>†</sup>	<.01* <sup>†</sup>	.02* <sup>†</sup>	<.001* <sup>†</sup>	<.01* <sup>†</sup>	.99 <sup>†</sup>	.93 <sup>†</sup>

Note.—F = female, M = male.

\*  $P < .05$

† Data are shown in Figures 2–4.

**Table E2: Welch T Test Results of Histologic Features**

Finding	Control				NAFLD Mice				P Value			
	1 Wk	12 Wk	24 Wk	48 Wk	1 Wk	12 Wk	24 Wk	48 Wk	1 Wk	12 Wk	24 Wk	48 Wk
ALT (IU/L)	8.6 ± 1.5	28.7 ± 8.4	23.5 ± 5.1	18.2 ± 2.7	12.4 ± 1.3	125.5 ± 64.1	151.6 ± 48.8	177 ± 67.1	.003	<.001	<.001	<.001
Steatosis grade (0–3)	0	0	0	0	0.8 ± 0.4	2.6 ± 0.7	3 ± 0	3 ± 0	.004	<.001	0	0
Steatosis location (0–3)	0	0	0	0	1.4 ± 0.9	2.6 ± 1.1	3 ± 0	3 ± 0	.008	<.001	0	0
Microvesicular steatosis (0–1)	0	0	0	0	0.2 ± 0.4	0.9 ± 0.4	1 ± 0	1 ± 0	.35	<.001	0	0
Fibrosis stage (0–4)	0	0	0	0	0.1 ± 0.1	0.2 ± 0.1	1.0 ± 0.7	3 ± 0.5	.04	<.001	.009	<.001
Lobular inflammation (0–3)	0.4 ± 0.5	0.3 ± 0.5	0.6 ± 0.5	0.5 ± 0.5	1 ± 0	0.5 ± 0.5	1.5 ± 0.8	2.5 ± 0.5	.04	.57	.04	<.001
Microgranulomas (0–1)	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.5	0	0	1	.54	.26	.26	.003
Large lipogranulomas (0–1)	0	0	0	0	0	0	0	0	1	1	1	1
Portal inflammation (0–1)	0	0	0	0	0	0	0	1	1	1	1	0

Ballooning (0-2)	0	0	0	0	0	0	0.6 ± 0.5	1.3 ± 0.5	1	1	.01	<.001
Acidophil bodies (0-1)	0	0	0.2 ± 0.4	0	0	0	0	0	1	1	.26	1
Pigmented macrophages (0-1)	0	0	0	0.2 ± 0.4	0	0	0	1 ± 0	1	1	1	<.001
Megamitochondria (0-1)	0	0	0	0	0	0	0	0.3 ± 0.5	1	1	1	.21
Mallory hyaline (0-1)	0	0	0	0	0	0	0	0.1 ± 0.4	1	1	1	.41
Glycogenated nuclei (0-1)	0	0	0	0	0	0	0	0	1	1	1	1

Note.—We define 0 as absolutely equal and 1 as absolutely different for the subgroups with zero standard deviations.