Supplementary Material to

"Multiparametric Magnetic Resonance for the non-invasive diagnosis of liver disease"

Supplementary Methods

To minimise motion artefacts from respiration and the cardiac cycle, Magnetic Resonance (MR) data were acquired during expiratory breath-hold and with ECG-gating. This required subjects to repeatedly hold their breath and lie still for 12 - 14 seconds, which was comfortably managed by all participants. These are standard techniques for cardiac data acquisition.[1]

Hepatic T1 mapping for fibrosis quantification

Image acquisition

A T1 relaxation time map was acquired using the Shortened Modified Look Locker Inversion recovery (shMOLLI) sequence[2] in a transverse plane through the right lobe of the liver and the spleen. This method has been developed by our centre to determine extracellular fluid content in parenchymal tissue, and can detect even subtle changes of 5%, which are not quantifiable using other imaging techniques. A subject–dependent frequency adjustment was carried out during end-expiration. The ShMOLLI sequence samples the T1 recovery curve using single-shot steady state free precession (SSFP) acquisitions using the following parameters:

TR 2.14ms, TE 1.07ms, flip angle of 35°, field-of-view optimised per patient, acquisition matrix 192x134-160, depending on patient, with GRAPPA acceleration of 2 with 24 reference lines, yielding a typical interpolated voxel size 0.9 x 0.9 x 8mm. Images were acquired 200ms after the ECG R-wave and the total time for each SSFP acquisition between 169 and 197ms, depending on the number of phase encoding steps. The variable acquisition

parameters fall in the ranges used in myocardial T1 mapping at 1.5T, with associated inter-individual coefficient of variation for normal myocardial T1 of 2.2%,[3] well within the inter-subject coefficient of variation measured in normal volunteers here of 7%.

Region of interest placement

A single Region of Interest (ROI) in segment 8 of the liver from where most biopsies are taken was selected for each patient. There were 4 important considerations in the choice of the ROI other than the placement in segment 8.

(a) As each acquisition generates an R^2 map for the fit of signal intensity to the exponential recovery curve the ROI was chosen in an area where R^2 was $\geq$ 99% (which was the case in all patients),

(b) The ROI was placed approximately halfway between the porta hepatis and the liver surface in order to avoid interference from the fluid filled structures in the porta hepatis and subcutaneous tissue or air close to the liver surface,

(c) The ROI was placed so as to avoid visible bile ducts and blood vessels,

(d) The ROI was placed in an area that corresponded to good quality images in the T2* map in order to allow T1 and T2* quantification in the same ROI.

Supplementary Figure 1 illustrates the ROI placement.

Hepatic ^{1}H MRS for steatosis quantification

^{1}H MRS data acquisition

A calibration pulse sequence was used to determine the optimum water suppression pulse scaling factor to better characterise the lipid peak at 1.3ppm. Five acquisitions were obtained per breath-hold with ECG-triggering to minimise noise from variations in arterial blood flow in the liver. As with the heart, a TR of 2 seconds allowed for complete relaxation of the lipid

signal between successive RF pulses. This required subjects to hold their breath and lie still for 12 - 14 seconds. Improved Signal to Noise Ratio (SNR) in the liver due to less motion artefact compared to the heart meant that fewer datasets were required, so only five breath-holds were used – 4 with water suppression 'on' for lipid data, and one with water suppression 'off' to determine the water signal. Spectroscopy parameters were otherwise similar to the cardiac setup (TE 10ms; mixing time 7ms; 1024 points acquired at a bandwidth of 2000Hz; scan frequency 1.3ppm for water-suppressed spectra and 4.7ppm for water-unsuppressed spectra; TR 2s for water-suppressed data and 4s for water-unsuppressed data). Signals from different coil elements in each breath-hold were combined, and individual spectra phase- and frequency- corrected prior to summation. Supplement Figure 3 illustrates examples of water suppressed and unsuppressed spectra.

Voxel location

Localiser images were obtained during end-expiratory breath-holds in the transverse, coronal and sagittal planes. A first voxel of interest was placed in the lateral aspect of the right lobe of the liver, avoiding visible vessels and the biliary tree (Supplementary Figure 2). The voxel was typically placed half way between the porta hepatis and the liver surface in order to avoid interference from the large fluid filed structures of the porta-hepatis and the subcutaneous tissue and air which lie close to the liver periphery.

Hepatic T2* mapping for quantification of iron deposition

A multi-gradient-echo acquisition with RF spoiling is used to calculate a T2* map of the liver. The same field-of-view as in the T1 mapping sequence is used, with a matrix size of 192x128-160, depending on patient, slice thickness of 3mm and 2x GRAPPA acceleration, with the same 200ms delay after the R-wave before acquisition. The image is acquired in nine

segments with a TR of 26.5ms and flip angle of 20°. Echo times are selected as far as possible such that the signals from fat and water are in phase (TE = 2.46, 7.38, 12.30, 17.22 and 22.14 ms). Fat-saturation and a double-inversion-recovery black blood preparation are used.

Region of interest placement

The region of interest in the T2* maps was chosen along the principles described above for the TI maps.

Collagen proportionate area quantification

Liver histology slides stained with Sirius Red were scanned using a Hamamatsu Nanozoomer 2.0 HT Digital Pathology System (Hamamatsu, Hamamatsu City, Japan) to produce high quality digital images of the whole biopsy sample and these images were analysed for collagen proportionate area[4] using ImageJ (U. S. National Institute of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, v1.47). The imaging analysis technique described by Calvaruso et al[4] was adapted for ImageJ by MP. The analysis was carried out jointly by MP and an expert liver histopathologist (LMW). The analysis was done blinded to the original histology report and the MRI assessment of fibrosis.

Supplementary References

1. Rial B, Robson MD, Neubauer S, Schneider JE. Rapid quantification of myocardial lipid content in humans using single breath-hold 1H MRS at 3 Tesla. *Magn Reson Med.* 2011;**66**(3):619–24.

2. Piechnik S, Ferreira V, Dall'Armellina E, et al. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. *J Cardiovasc Magn Reson.* 2010;**12**:69

3. Piechnik SK, Ferreira VM, Lewandowski AJ, et al. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5 T using ShMOLLI. *J Cardiovasc Magn Reson.* 2013;**15**:13

4. Calvaruso V, Burroughs AK, Standish R et al. Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient. *Hepatology.* 2009;**49**:1236-44.

Supplementary Tables

Supplementary Table 1. Baseline characteristics of 7 healthy volunteers on no regular medication and no known liver, cardiac or metabolic disease.

	Median / *Mean*	**IQR / *SD***	**Min value**	**Max value**
Male : Female	3 : 4			
Age (years)	39	12	27	55
Anthropometric data				
Weight (kg)	*59*	*9*	*47*	*73*
BMI (kg/m^2)	*20.9*	*2.4*	18.0	24.0

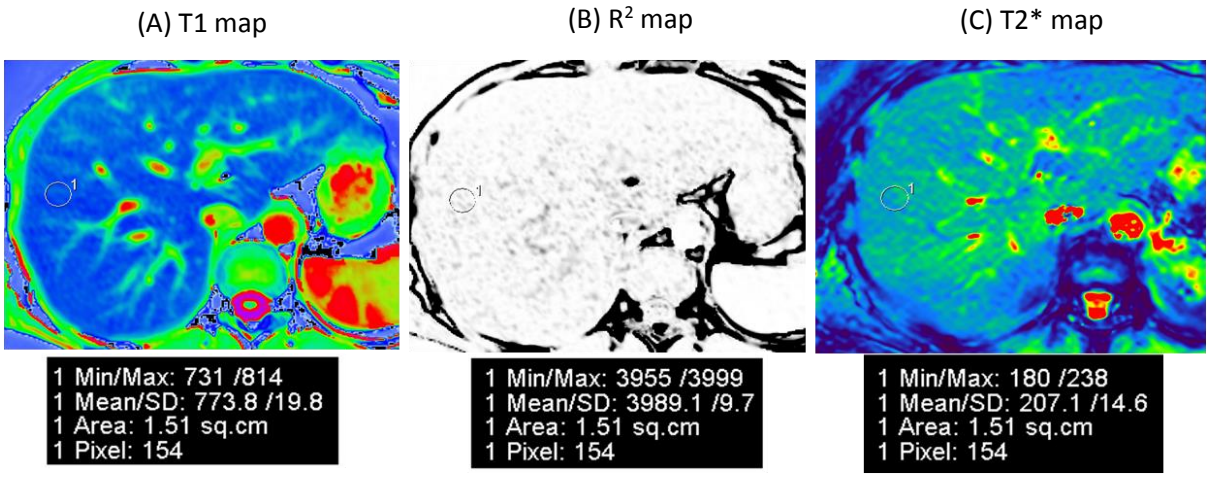
Values shown as *mean* ± *SD* for normally distributed variables *(italics)*, and median with IQR for non-normally distributed variables. Shapiro-Wilk test for normality used.
BMI; Body Mass Index, IQR: Inter Quartile Range, SD; Standard Deviation.

Supplementary Table 2. Trivariate weighted kappa statistic for the assessment of inter-observer agreement between pathologists for the evaluation of fibrosis steatosis and haemosiderosis of liver biopsies.

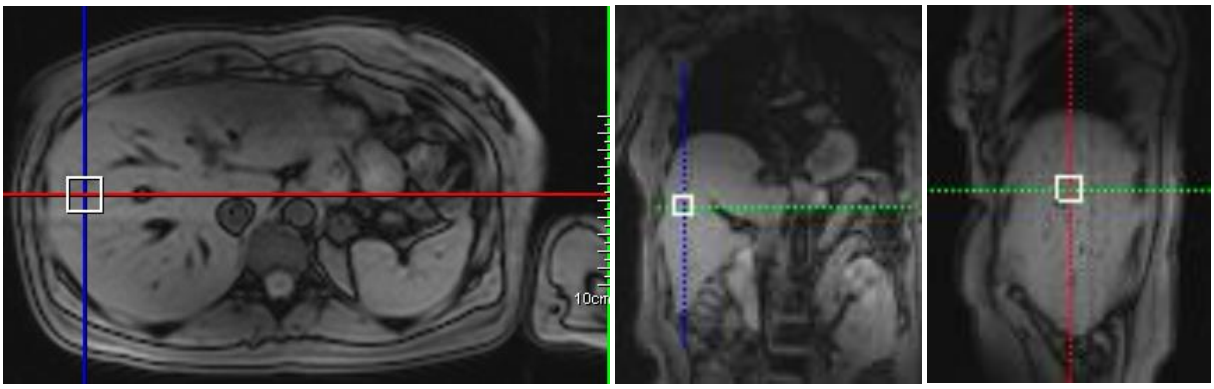
	Fibrosis Ishak (F0-6)	NAFLD Fibrosis stage (F0-4)	**Steatosis** Steatosis Score (0-3)	% hepatocytes with lipid inclusions	**Iron** Iron grading of Perls' stained biopsies (0-4)
Trivariate weighted kappa statistic	0.58	0.51	0.72	0.71	0.58

Each pathologist was a specialist in hepatobiliary disease, and reported the biopsy without the aid of any clinical or MR data and independently of each other.

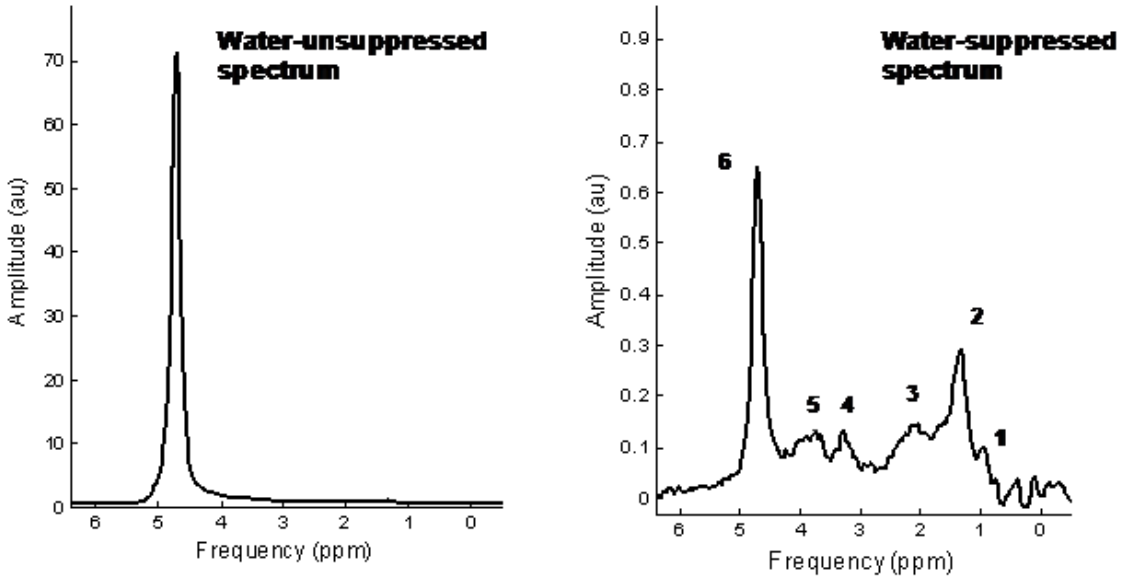
Supplementary Figures



Supplementary Fig. 1. Region of interest placement in T1 and T2* maps. The region of interest was placed on the same location in segment 8, in the T1, R^2 (quality assurance component of T1 mapping) and T2* maps as illustrated here. The number of pixels, area, minimum, maximum, mean and standard deviation for each Region of Interest are automatically read by the scanner console software. The mean T1 and T2* value for each ROI was used in the final analysis.

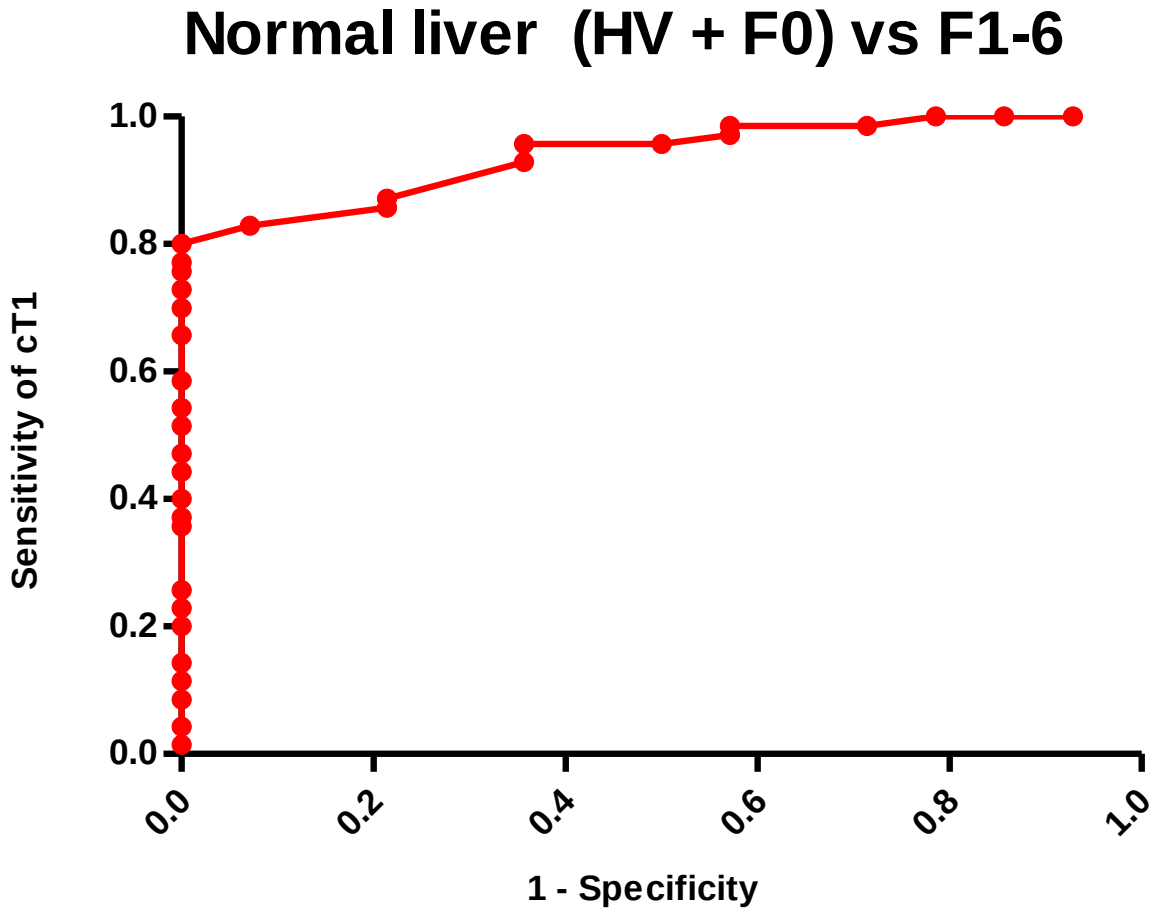


Supplementary Fig. 2. ^{1}H MRS voxel location. Localiser images were obtained in the 3 orthogonal axes and the voxel was placed in segment 8 in an area avoiding large blood vessels and bile ducts.

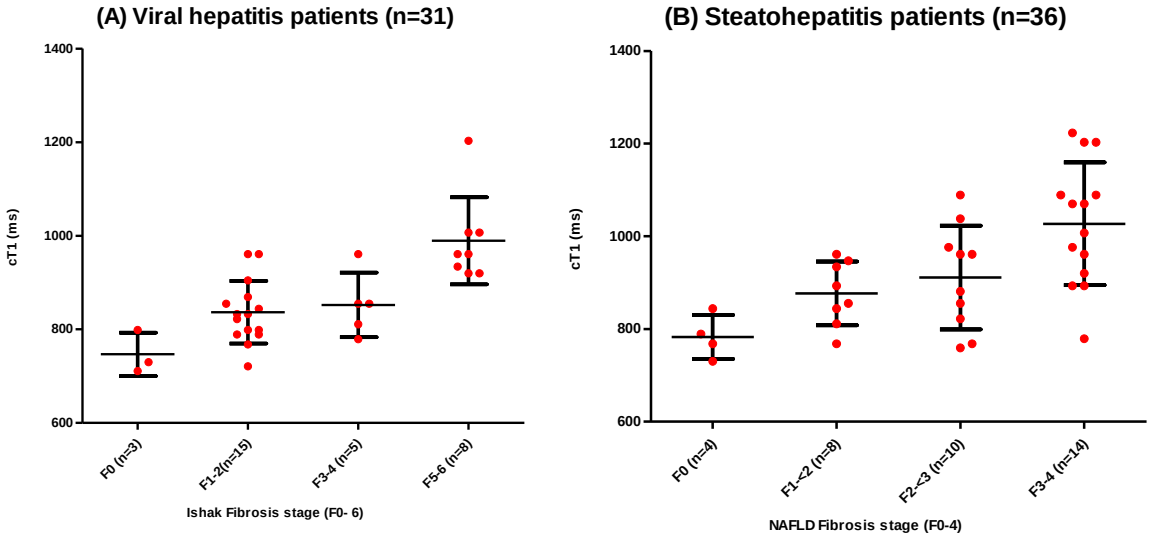


Supplementary Fig. 3. ^{1}H MRS resonances of major liver metabolites. Typical water unsuppressed and water suppressed spectra are shown. Each peak in the water unsuppressed spectrum represents the relative abundance of the metabolites in the liver as listed below. Hepatic lipid content is calculated as (area under peak 2) ÷ (area under water peak in unsuppressed spectrum) x 100%.

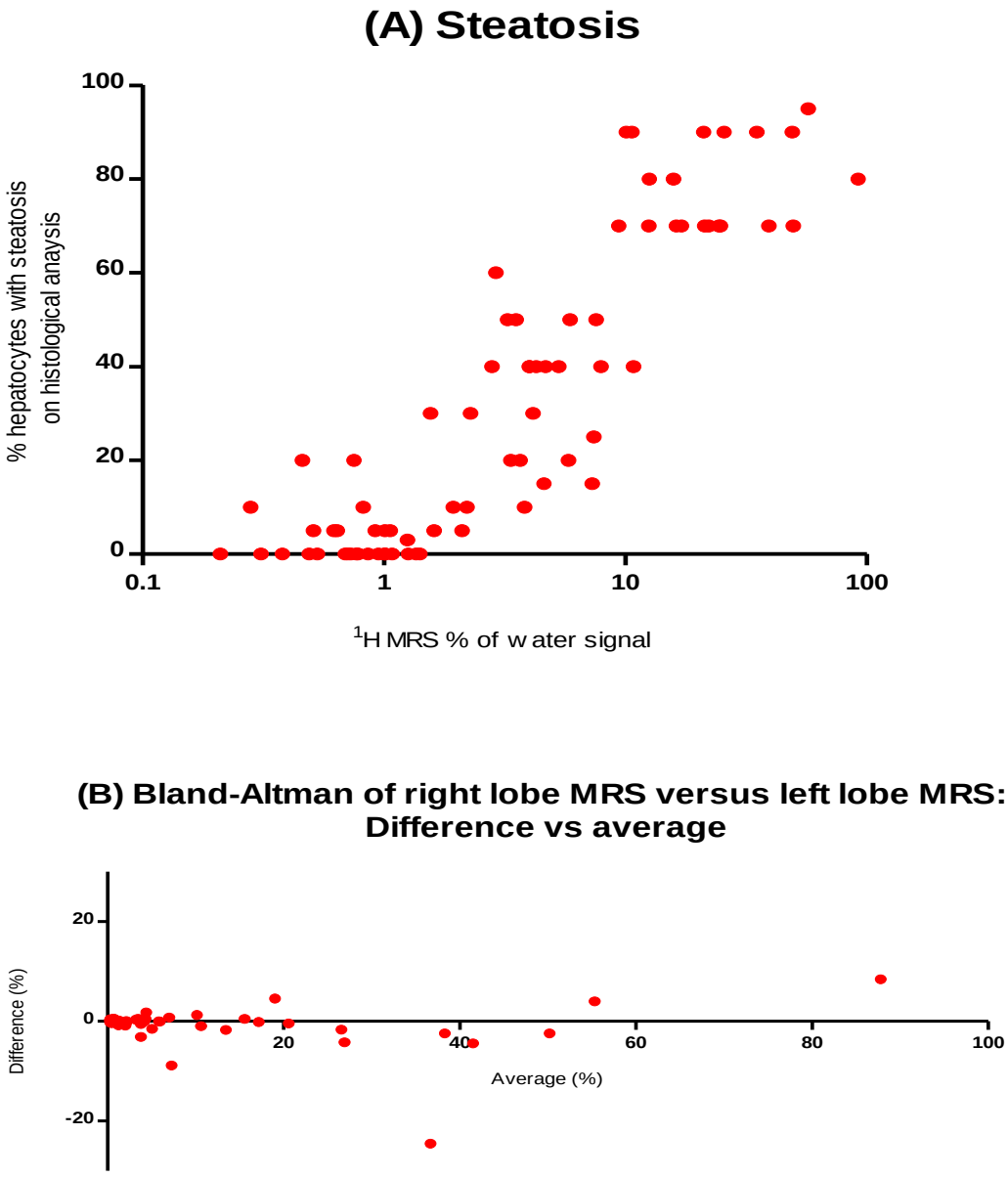
1. Triglyceride terminal CH3 (0.9ppm)
2. Methylene $(-CH_2-)_n$ (1.3ppm)
3. –CH2-CH=CH- (2ppm)
4. Choline (3.2ppm0
5. Glycogen (3.6-4ppm)
6. Residual water (4.7ppm)



Supplementary Fig. 4. Receiver operator characteristic curve (ROC) for iron corrected T1 (cT1) measurements for diagnosing the presence of any fibrosis (F≥1). The MR data of healthy volunteers are included in the analysis. AUROC is 0.94 (95% CI 0.89 – 0.99).



Supplementary Fig. 5. cT1 for the evaluation of fibrosis in patients with viral hepatitis and steatohepatitis. (A) There was excellent correlation in cT1 with increasing Ishak fibrosis stage (r_s = 0.76, p < 0.0001, 95% CI 0.55 to 0.88) in 31 patients with viral hepatitis. (B) In patients with steatohepatitis (n=36) cT1 was compared to the average NAFLD fibrosis stage from three blinded pathologists. Again, cT1 correlated well with increasing degrees of fibrosis (r_s = 0.62, p < 0.0001, 95% CI 0.36 to 0.79). This is the first non-invasive test to determine the presence of any fibrosis in fatty liver disease (AUROC 0.90, 95% CI 0.79 – 1.01). cT1, iron corrected T1; NAFLD, Non-Alcoholic Fatty Liver Disease.



Supplementary Fig. 6. Correlation of ^{1}H MRS HLC and quantitative histology score of steatosis and Variance of HLC between the right and left liver lobes as assessed by ^{1}H MRS. (A) ^{1}H MRS measurement of Hepatic Lipid Content showed excellent correlation with quantitative histologic assessment of steatosis expressed on a continuous scale as % of hepatocytes with lipid inclusions (r_s = 0.88, p < 0.0001, 95% CI 0.81 to 0.92). (B) Bland-Altman plot showing little variance between the degree of steatosis in the right and left liver lobes suggesting that typically steatosis is uniformly distributed.