Hepatic Lipidosis and Fibrosis in Obese, Dysmetabolic, and Diabetic Non-Human Primates Quantified by Noninvasive Sonography

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INTRODUCTION

Liver biopsy is the gold standard for clinical diagnosis of nonalcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH), however, the procedure is invasive, costly and prone to sampling error because of heterogeneous pathology in the liver. We present a noninvasive sonographic method coupled with software image analysis to quantify hepatic lipidosis in correlation with multiple dysmetabolic and liver fibrosis biomarkers in 36 cynolmolgus monkeys with normal, obese, dysmetabolic and diabetic metabolic phenotype. Both the hepatic/renal echo-intensity ratio (H/R = 1.69 ± 0.12 vs 1.36 ± 0.09) and hepatic echo-intensity attenuation rate (HA = 0.41 ± 0.07 vs 0.17 ± 0.04 MHz/cm) were significantly higher in the obese (n=14) compared to control (n=22) monkeys. Ultrasound indices highly correlated with multiple metabolic risk factors such as hyperlipidemia, liver fibrosis indices, body mass index (BMI), Alanine/Aspartate Transaminase (AST/ALT), diabetes (BARD) score, fibrosis-4 (FIB4), and AST to platelet ratio index (APRI).

METHODS

Ultrasound imaging was performed with ProSound SSD-3500SX (Hitachi Aloka Medical, Ltd. Tokyo, Japan) with a 3.5 – 5 MHz convex transducer by 2 different radiologists blinded to the metabolic status of the animals during scanning and imaging analysis. All the instrument settings, including "gain", "depth", "time-gain compensation", etc. were fixed for each measurement. Representative images of the liver, kidney and surrounding organs were captured at different defined angles. All digitized ultrasound images were analyzed by 2 radiologists involved in scanning, using Image J software (version 1.41, NIH, Bethesda, MD).

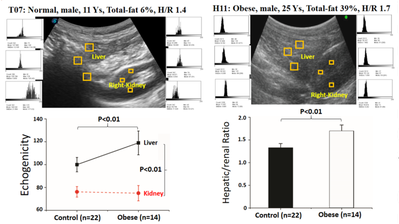

Fig. 1: Hepatic/renal echo-intensity ratio. Representative ultrasound images show selected regions of interest (ROI, Square) for measurement of echo-intensity in the liver parenchyma and kidney cortex regions from a representative normal (left) and obese (right) NHP (top) and average echo-intensity in the liver parenchyma and kidney cortex (left) and hepatic/renal ratio (H/R, right) in the control and obese groups (bottom).

RESULTS

Liver parenchyma from control NHP (**Figure 1**, left) had homogeneous echo texture with similar or a slightly higher echogenicity when compared to the parenchyma of the kidney cortex and spleen. In contrast, in obese NHPs we observed fatty liver **Figure 1,** right) with a greater echogenicity (bright liver) than the kidney cortex and spleen parenchyma. As shown in **Figure 1**, the average echo-intensity for the liver parenchyma (but not the kidney cortex) was significantly higher, with a greater H/R ratio (right) in the obese than control group. **Figure 2** shows the comparison of echogenicity and echo-intensity attenuation rate between control and obese subjects using near and far field liver ultrasound images. Linear regression analysis revealed a strong positive correlation (**Figure 3**) between the H/R ratio with FIB4 (A), APRI (B) and BARD (C), but not with CK-18 (D). Animals with high H/R ratio had significantly higher liver fibrosis indexes except for CK-18, and lower platelet counts than those with low H/R ratio (**Table 1**). Furthermore, at a defined threshold for each risk factor, the univariable and multivariable analysis revealed that BMI, total fat, FBG, HbA1c, TG, HA, as well as the liver fibrosis indices except for CK-18 and platelet counts were significantly associated with H/R ratio (**Table 2**).

Table 2. Univariable and multivariable analysis of the risk factors associated with hepatic/renal eco-intensity ratio (H/R)

	OR	95% CI for OR	*p* value
General Characterization			
Age (>10 yr)	1.4	0.3-5.6	0.636
BW(> 9.3 kg)	1.9	0.5-7.1	0.366
BMI (> 13.5 kg/m^2)	4.1	1.0-16.6	**0.049**
WC (> 45 cm)	1.6	0.4-5.9	0.503
Total-fat (> 20 %)	6.5	7.2-5.7	**0.017**
Trunk-fat (> 20 %)	3.0	0.8-11.9	0.065
FBG (> 85 mg/dL)	1.7	1.0-2.4	**0.047**
HbA1c (> 6 %)	1.7	1.0-2.5	**0.037**
CHO (> 200 mg/dL)	1.2	0.3-4.4	0.821
HDL (< 35 mg/dL)	1.0	0.3-3.6	0.940
LDL (> 140 mg/dL)	0.8	0.4-1.0	0.506
TG (> 150 mg/dL)	3.8	2.9-4.8	**0.030**
Liver Fibrosis Indices			
FIB4 (> 3.6)	19.8	3.2-120.0	**0.001**
BART (> 2)	27.9	3.0-257.3	**0.003**
APRI (> 18)	5.0	1.2-20.9	**0.028**
CK-18 (> 13 ng/mL)	0.9	0.2-3.6	0.881
ALT (> 40 (male), 31 (female) IU/L)	1.2	1.0-1.9	**0.041**
AST (> 37 (male), 31 (female) IU/L)	3.2	1.2-5.2	**0.000**
Platelet count (> 310 10^9/μL)	0.3	0.1 – 0.2	0.091
Liver Ultrasound			
HA (> 0.19 MHz/cm)	13.0	2.6-65.2	**0.002**

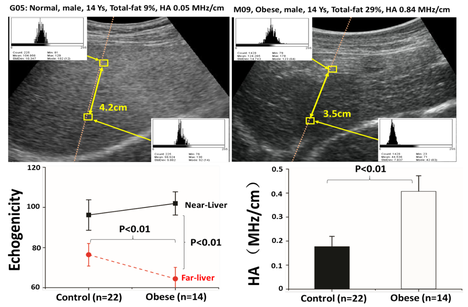

Fig. 2 Representative ultrasound images show selected regions of interest at the near and far field of the liver parenchyma from a representative normal (left) and obese (right) NHP (top panel) and average echo-intensity of near and far liver parenchyma, respectively (left) and echo-intensity attenuation rate (HA, right) in in the control and obese groups (bottom panel)

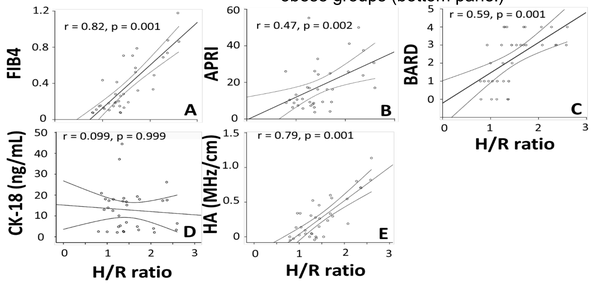

Fig. 3 Correlation between hepatic/renal echo-intensity ratio (H/R) with liver fibrosis indices: A. Fibrosis-4 (FIB4); B. AST to Platelet Ratio Index (APRI); C. BMI, AST/ALT ratio, Diabetes score (BARD); D. Serum cytokeratin 18 fragment (CK-18); and E. Hepatic echo-intensity attenuation rate (HA)

Table 1: Characterization of monkeys for hepatic/renal echo-intensity ratio (H/R)

	H/R < 1.4 (n=20)	H/R > 1.4 (n=16)	*p* value
General Characterization			
Age (yr)	12.1±0.8	12.6±1.3	0.359
BW(kg)	9.5±0.6	9.1±0.8	0.334
BMI (kg/m^2)	13.6±0.6	13.8±0.9	0.100
WC (cm)	44.8±1.9	46.7±3.0	0.292
Total-fat (%)	16.5±2.5	18.8±3.4	0.276
Trunk-fat (%)	19.4±3.0	22.1±3.9	0.281
FBG (mg/dL)	111.1±14.2	119.9±12.6	**0.049**
HbA1c (%)	5.1±1.0	5.9±0.9	0.117
CHO (mg/dL)	116.4±6.5	108.4±5.5	0.182
HDL (mg/dL)	47.0±3.2	49.8±3.0	0.334
LDL (mg/dL)	46.6±4.3	43.8±5.3	0.342
TG (mg/dL)	100.0±17.2	110.6±17.6	0.051
Liver Fibrosis Indices			
FIB4	0.25±0.0	0.61±0.1	**0.000**
BARD	1.5±0.3	3.2±0.2	**0.000**
APRI	10.5±1.2	28.1±3.2	**0.000**
CK-18 (ng/mL)	13.8±2.7	11.5±2.1	0.529
ALT (IU/L)	38.2±5.2	59.1±7.9	**0.022**
AST (IU/L)	34.8±3.9	65.0±7.3	**0.001**
Platelet (10^9/μL)8	340.8±17.1	270.9±37.4	**0.039**
Liver Ultrasound			
HA (MHz/cm)	0.18±0.04	0.40±0.06	**0.005**

SUMMARY

- **Noninvasive ultrasonography in combination with biomarkers can be used routinely as an aid for detection and monitoring of NAFLD/NASH disease progression**
- **We used the hepatic-renal echogenicity ratio to quantify the degree of hepatic lipidosis and fibrosis and found it could be well correlated to several relevant biomarkers, such as serum cytokeratin 18 and Fibrosis-4**
- **Further investigation and refinement of noninvasive sonographic techniques in NHPs will aid in the understanding and diagnosis of NAFLD/NASH in a human clinical setting**