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Ultrasound Derived Fat Fraction (UDFF) and MRI-PDFF of Adult Liver and Spleen: A Preliminary Observation

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1. Abstract

1.1. Objectives: The aim of the study was to assess ultrasound derived fat fraction (UDFF) and magnetic resonance imaging derived proton density fat fraction (MRI-PDFF) of the liver and spleen in adults with and without hepatic steatosis.

1.2. Methods: We prospectively measured UDFF and MRI-PDFF of the liver and spleen in 45 participants (22 men and 23 women, mean age 51y, age range 20-75y) after receiving the Institutional Review Board approval and written informed consent. Based on MRI-PDFF, participants were divided into normal liver group (MRI-PDFF <5%) or steatotic liver group (MRI-PDFF ≥5%). Differences in hepatic and splenic UDFF and MRI-PDFF between the two groups were examined by two-tailed t-test. The correlation of liver UDFF to MRI-PDFF was analyzed using linear regression. Diagnostic performance of UDFF and UDFF liver to spleen ratio (L/S ratio) in determining hepatic steatosis was tested using area under receiver operating characteristic curve (AUC).

1.3. Results: Liver UDFF and MRI-PDFF differed significantly between participants with (n=33) and without (n=12) NAFLD. No significant difference in UDFF or MRI-PDFF of the spleen was observed between participants with and without NAFLD. Liver MRI-PDFF was closely correlated with liver UDFF (R^2= 0.798) and UDFF L/S ratio (R^2= 0.897). AUC of UDFF and UDFF L/S ratio in determining ≥ mild hepatic steatosis was 0.913 and 0.985.

1.4. Conclusions: UDFF and UDFF liver to spleen ratio positively correlate with liver MRI-PDFF in quantifying fat content in the liver. Splenic UDFF and MRI-PDFF did not change following the development of hepatic steatosis.

2. Introduction

Currently, Nonalcoholic Fatty Liver Disease (NAFLD) has a prevalence of 30% in the United States [1] and is a leading cause of chronic liver disease worldwide [2]. There exists a strong association between NAFLD and metabolic syndrome. Metabolic syndrome is characterized by insulin resistance, hyperglycemia, hypertriglyceridemia, hypertension, and an increased waist to hip ratio or waist circumference. Individuals with these findings may warrant evaluation for NAFLD [3]. Individuals with NAFLD can also manifest features of chronic liver disease (ie. clubbing, palmar erythema, asterixis, spider angiomata, ascites, and/or gynecomastia) in addition to splenomegaly, cytopenia, and elevated ferritin levels [4]. As reported, liver fat content higher or equal to 10% is associated with a 10% increase in the odds ratio in the development of impaired glucose tolerance [1]. Therefore, it is clinically important to detect NAFLD in its early stages when fat accumulation in the liver is potentially reversible through weight loss and other healthy lifestyle measures that have been shown on histology to reverse fatty infiltration in the liver [5].

The gold standard for diagnosis of NAFLD is liver biopsy. This procedure has several limitations including high cost, sampling error, high inter-observer and intra-observer variation in pathology interpretation. Liver biopsy is also highly invasive, which risks complications such as bleeding and infection [6]. Serum biomarkers such as alanine aminotransferase (ALT) are not sufficiently sensitive in screening for hepatic steatosis as they may report normal in chronic liver disease [7]. Given the clinical value of detecting NAFLD in its

early stages and the limitations of current invasive diagnostic testing related to the disease, development of other non-invasive and efficacious options for screening, grading, and monitoring NAFLD is imperative.

Among non-invasive imaging techniques, Computed Tomography (CT) has demonstrated low sensitivity and accuracy in the diagnosis of mild steatohepatitis [8, 9]. Additionally, CT exposes the patient to ionizing radiation making it unsuitable for long term repeated usage in NAFLD screening and monitoring. Magnetic resonance imaging derived proton density fat fraction (MRI-PDFF) is currently the imaging modality of choice in the diagnosis of NAFLD due to its quantitative nature, reliable accuracy, acceptable sensitivity and specificity, and lack of radiation exposure. There is significant agreement between MRI-PDFF values and histologically confirmed NAFLD and nonalcoholic steatohepatitis (NASH) [10]. Limitations of MRI-PDFF include lack of access for some medical facilities, particularly in rural areas; clinical contraindications such as patients with implantable medical devices or claustrophobia; and high cost. These factors limit MRI use as a first-line diagnostic test in screening for hepatic steatosis, evaluating treatment effectiveness, and monitoring disease progression of NAFLD.

Conventional B-mode ultrasound relying on relative echogenicity of liver parenchyma compared to kidney cortex has poor sensitivity and specificity and thus, has not been useful in diagnosing NAFLD [11]. In addition, it has high intra-observer and inter-observer variability. One study demonstrated inter-observer agreement for grading hepatic steatosis into normal, mild, moderate, and severe steatosis was between 53-62%. The same study demonstrated that intra-observer agreement for readings ranged between 55-68% [12]. Ultrasound derived quantitative biomarkers including attenuation coefficient, backscatter coefficient, and speed of sound have recently been developed for detection of NAFLD. However, the sensitivity, specificity, and robustness of these imaging biomarkers are still under clinical investigation [13].

Ultrasound derived fat fraction (UDFF), a newly developed ultrasound technique for noninvasive quantification of hepatic steatosis during routine abdominal ultrasound exam, is an ideal test for screening and monitoring NAFLD progression [14]. UDFF quantifies the percentage (%) of hepatic steatosis through specific software that simultaneously measures both attenuation coefficient and backscatter coefficient in the liver parenchyma [14]. A literature review on UDFF revealed an absence of investigation regarding measurement of UDFF in the spleen of adults with and without NAFLD. The aim of this study was to investigate the difference in UDFF and MRI-PDFF of the liver and spleen in participants with and without NAFLD and to evaluate the correlation between MRI-PDFF and UDFF findings in adult livers.

3. Material and Methods

3.1. Ethics

The Institutional Review Board of Rocky Vista University approved the study (2019-0009) and all participants provided written informed consent prior to participation. The study protocol was confirmed to meet the ethical guidelines of the declaration of Heisinki by the university.

3.2. Participants

3.2.1. Inclusion criteria: age 20y or older; alcohol assumption < 20 g/day; ability to provide informed consent; ability to fast 6 hours; ability to tolerate ultrasound and MRI scans; clinical risk for, or known, nonalcoholic fatty liver disease.

3.2.2. Exclusion criteria: age < 20y; history of viral hepatitis (hepatitis virus a, b, and c), chemotherapy, major liver interventions (surgery, ablation), hepatic cell carcinoma, liver metastatic lesions, systemic malignancies (lymphoma, leukemia, systemic lupus), focal or diffuse splenic lesions, sickle cell disease, splenic arteriovenous malformation, splenic infarction, or splenic surgery.

3.3. Ultrasound Derived Fat Fraction (UDFF)

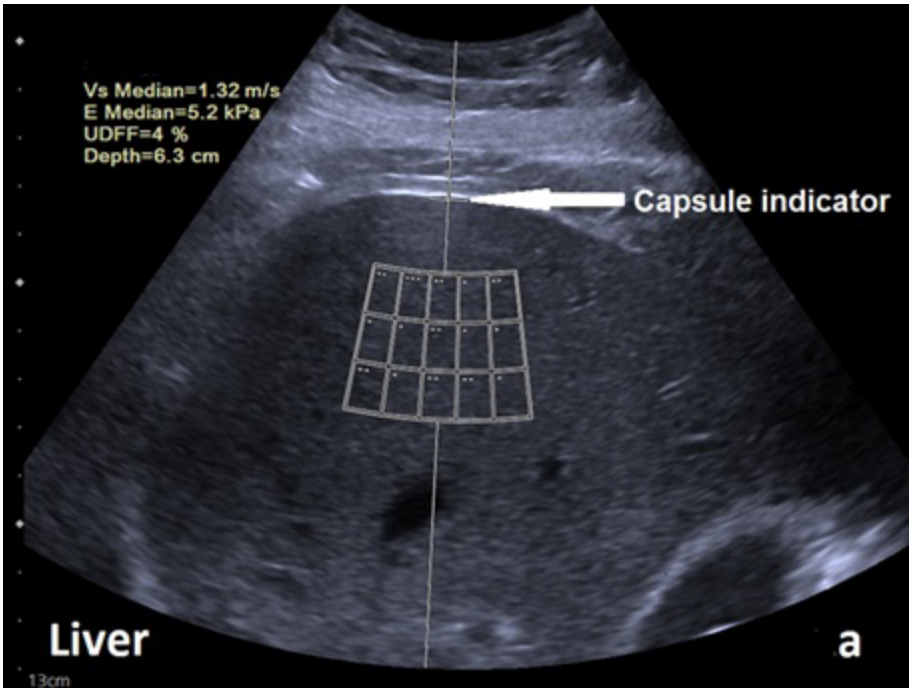
UDFF is a newly developed ultrasound biomarker that assesses hepatic steatosis by estimating the frequency-dependent attenuation coefficient (AC, dB/cm/MHz) and backscatter coefficient (BSC, 1/cm-Sr) through processing acoustic radiofrequency (RF) signals returned from the liver tissue. There are different characteristic impedances between accumulated fat vesicles in hepatocytes compared to normal liver tissue [14]. Positive correlation has been demonstrated between the amount of liver fat content and UDFF values, as a combination between AC and BSC assessments [14]. Additionally, MRI-PDFF also correlates well to individual AC and BSC using liver histology as the reference standard [15]. A good to excellent intra- and inter-observer reliability in measuring liver UDFF has been reported [16].

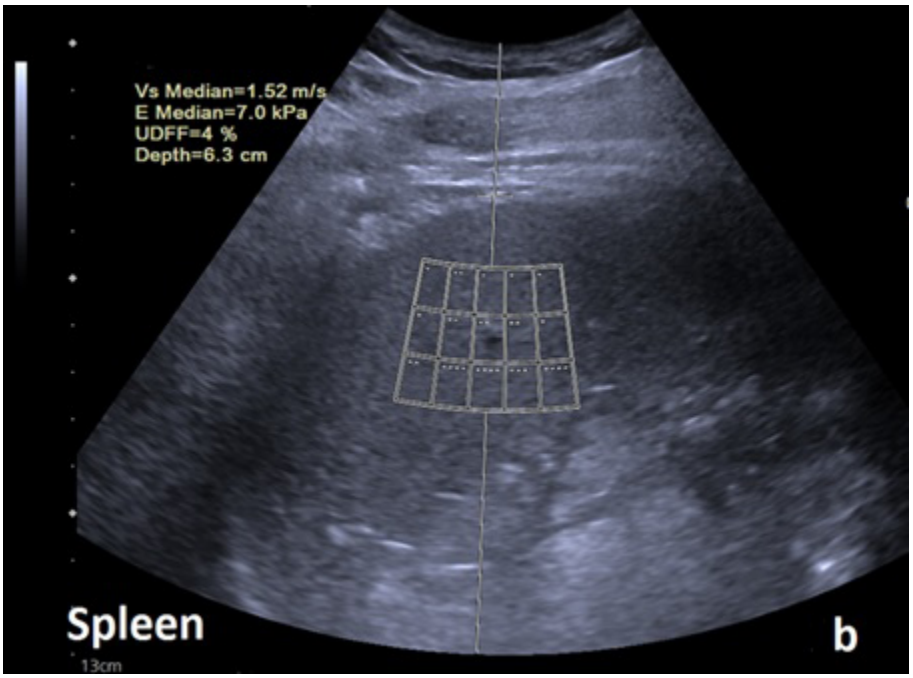
An Acuson Sequioa ultrasound scanner equipped with a curvilinear transducer (5C1, bandwidth 1.0-5.7 MHz, center frequency 3.0 MHz, Siemens Healthineers, Issaquah, WA) was used to acquire B-mode image and measure UDFF of the liver and spleen following manufacturer recommended machine settings and scanning protocols [16].

After fasting 6 hours, the participant was placed in the left lateral recumbent position to image the liver and then in the right lateral recumbent position to image the spleen. The liver and spleen were scanned with B-mode ultrasound to assess organ size, vasculature, and the presence of cystic or solid lesions. A breath-holding maneuver (holding breath for 5 seconds at the end of expiration during a normal breath cycle) was used to minimize patient movement and its associated out-of-plane motion artifact while measuring UDFF of the liver and spleen. A large region of interest (ROI, 3.0 cm × 3.0 cm, laterally by axially) for measuring liver UDFF was placed in the parenchyma 1.5 cm from the hepatic capsule using a capsule indicator (Figure 1). The ROI for measuring UDFF of the spleen was place in

the mid-portion of the spleen where the parenchyma appears larger than the size of ROI (Figure 2). Motion artifacts, multiple reflections, acoustic shadowing from ribs, liver (spleen) capsule, and major intrahepatic vessels were excluded from ROI for measuring UDFF. The UDFF was measured 5 times in each organ (liver and spleen) and the average of these UDFF values was used for analysis. UDFF liver to spleen ratio (UDFF L/S ratio) was calculated by the mean UDFF value of the liver divided by the mean UDFF value of the spleen.

A senior operator (J.G.) with 30 years of experience in abdominal ultrasound and 10 years in ultrasound elastography performed all ultrasound scans.





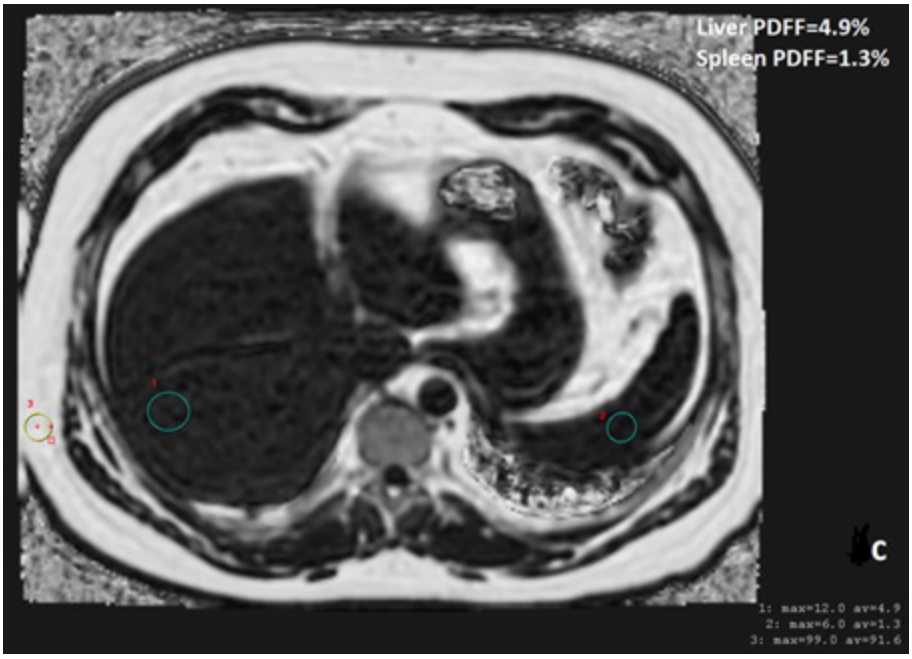
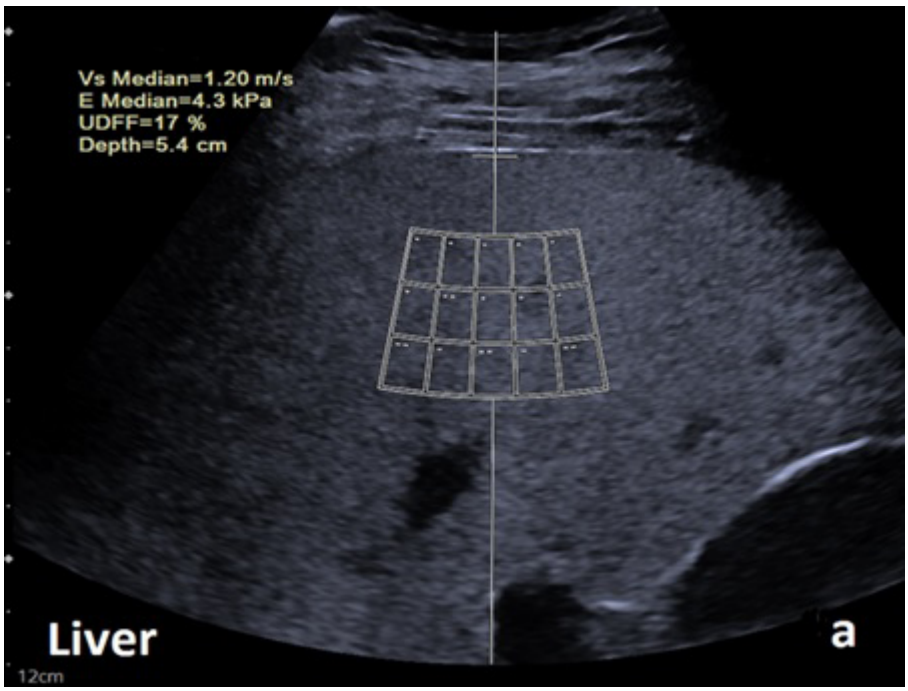


Figure 1a-c: Ultrasound derived fat fraction (UDFF) was measured in a 35y healthy man without history of alcohol intake or liver disease of any kind. UDFF of the liver and spleen measures 4% (1a) and 4% (1b), respectively. UDFF liver to spleen ratio of this participant is 1. His average liver and spleen MRI-PDFF measures 4.9% and 1.3%, respectively (1c).



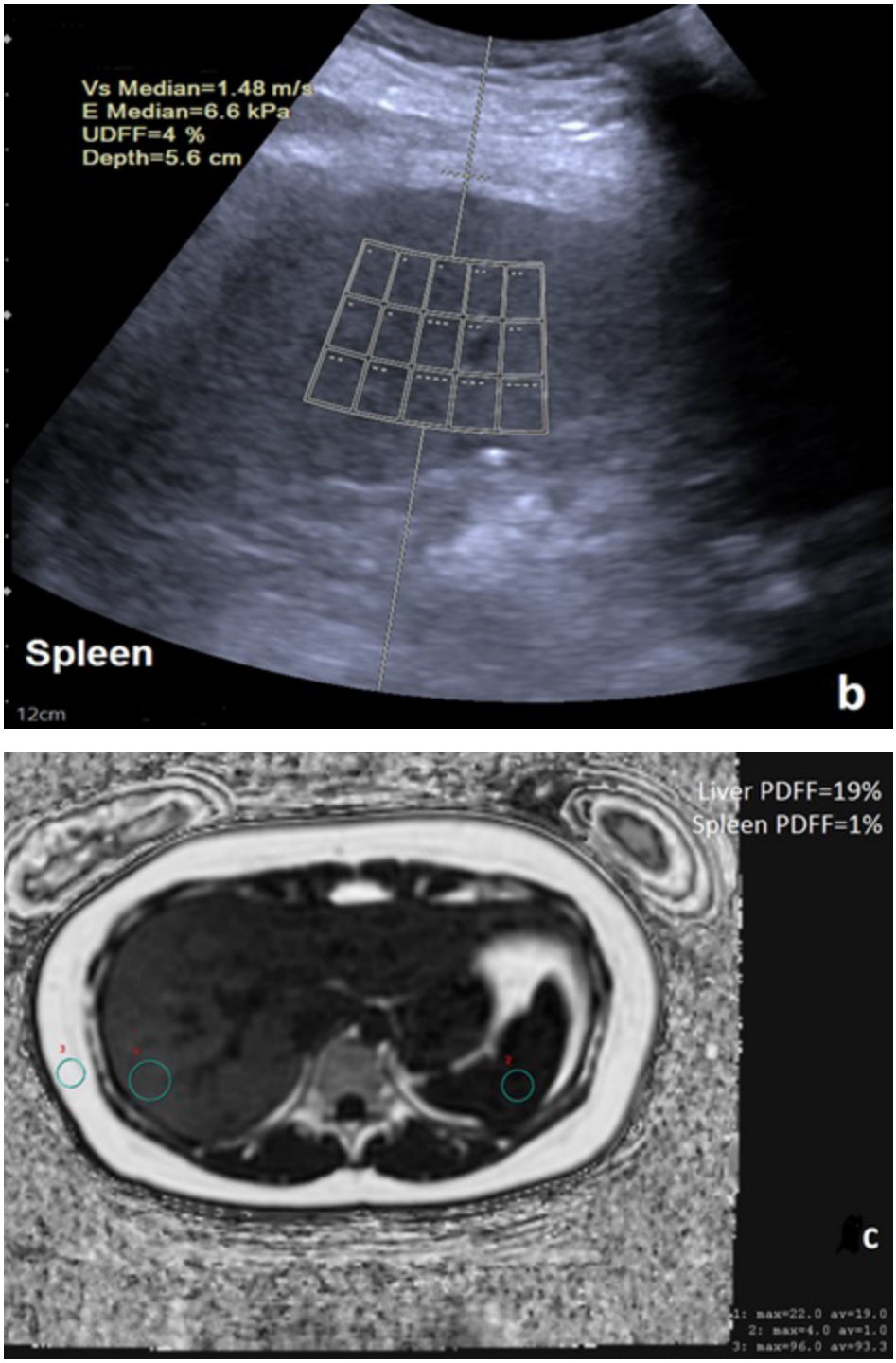


Figure 2a-c: Ultrasound derived fat fraction (UDFF) was measured in a 40y woman with long history of high cholesterol. UDFF of the liver and spleen measures 17% (2a) and 4% (2b), respectively. UDFF liver to spleen ratio of this participant is 4.25. Her average liver and spleen MRI-PDFF measures 19% and 1.0%, respectively (2c). Liver MRI-PDFF 19% suggests a moderate hepatic steatosis.

3.4. Magnetic resonance imaging derived proton density fat fraction (MRI-PDFF)

Participants fasted 6 hours prior to their MRI scan. A 3.0T MRI scanner equipped with a 32- channel phased–array (Discovery MR 750, GE Healthcare, Milwaukee, WI) was used to perform MRI-PDFF. Breath-holding sequences of 3D multiple fast spoiled gradient echo using a multi-point Dixon technique (IDEAL IQ) were acquired in the axial plane. Imaging parameters included: field of view =48 cm, matrix =148 x 148, 80 slices with thickness = 6 cm, flip angle= 3 degree, auto-calibrating reconstruction for cartesian imaging outer acceleration factor of 2, bandwidth = ±100kHz, repetition time= 4.8ms, 6 echo times acquired in 2 acquisitions. By normalizing the signal from fat protons to the signal from both fat and water protons, the MRI-PDFF showed the percentage of fat content in the liver (Figure 1c, Figure 2c) [17]. MRI-PDFF was measured nine times in each liver and five times in each spleen. The average of liver and spleen MRI-PDFF values were used for analysis.

3.5. Statistical Analysis

All variables including the age, body mass index, depth from the skin to liver (spleen) capsule, MRI-PDFF, and UDFF are expressed by the mean and Standard Deviation (SD). Based on the MRI-PDFF determined fat content of the liver, all participants were divided into normal liver (MRI-PDFF < 5%) group or steatotic liver (MRI-PDFF ≥ 5%) group. Differences in all variables between the two groups were examined by two-tailed t test. The correlation of liver MRI-PDFF to liver UDFF and UDFF L/S ratio were analyzed by linear regression [18]. The diagnostic performance of UDFF in determining hepatic steatosis was examined by area under ROC (AUC). A P value less than 0.05 was considered statistically significant. All analyses were conducted using a commercial software (SPSS version 28.0, IBM).

4. Results

From January 2021 to March 2022, we successfully performed UDFF and MRI-PDFF of the liver and spleen in 45 adults (8 were referred by a hepatologist and 37 were local volunteers) (Table 1). Based on MRI-PDFF, the 45 participants were divided into the normal liver group (12 participants with MRI-PDFF <5%) or steatotic liver group (33 participants with liver MRI-PDFF ≥5%, included 13 livers with MRI-PDFF 5-12%, 15 livers with MRI-PDFF 12.1-20%, and 4 livers with MRI-PDFF >20%) [17, 19]. The participants' demographic information (age, gender, body mass index), the distance from skin to the liver capsule, the distance from the skin to the spleen capsule, the distance from the skin to the center of ROI, UDFF, and MRI-PDFF of the liver and spleen are listed in Table 1. Differences in liver UDFF, liver MRI-PDFF, and UDFF L/S ratio between the normal liver and steatotic liver groups were significant ($p< 0.001$) whereas differences in spleen UDFF and spleen MRI-PDFF between the two groups were not significant ($p> 0.05$). There were no significant differences in age, BMI, distance from the skin to liver or spleen capsule, or the distance from the skin to the center of ROI for measuring UDFF in the liver or spleen between the two groups ($p> 0.05$) (Table 1).

The liver MRI-PDFF was closely correlated with liver UDFF ($R^2=0.798$, $p < 0.001$, Figure 3a) and UDFF L/S ratio ($R^2=0.897$, $p <0.001$, Figure 3b). AUC of UDFF and UDFF L/S for determining mild hepatic steatosis was 0.913 and 0.985, respectively (Table 2).

Table 1: Liver and spleen parameters between participants with and without hepatic steatosis

Parameters	Normal livers	Steatotic livers	P value*
Number of participants	12	33	
Men/women	7/5	17/16	
Age (y)	48±17	53±13	0.32
Body mass index (kg/cm^2)	30.62±7.78	32.7±8.13	0.29
Skin to liver capsule (cm)	2.89±0.59	3.16±0.33	0.39
Skin to spleen capsule (cm)	2.78±0.67	3.02±0.71	0.34
Skin to ROI (center) in liver (cm)	5.55±0.50	5.88±0.62	0.45
Skin to ROI (center) in spleen (cm)	5.58±0.52	5.87±0.55	0.49
Liver MRI-PDFF (%)	4.07±0.57	14.19±5.55	< 0.001
Spleen MRI-PDFF (%)	1.49±0.33	1.48±0.29	0.88
Liver UDFF (%)	5.16±1.84	15.48±5.11	< 0.001
Spleen UDFF (%)	3.16±0.56	3.11±0.59	0.8
UDFF L/S ratio	1.64±0.40	5.403±1.73	< 0.001

Note: * p value is based on two-tailed t-test; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; UDFF, ultrasound derived fat fraction; L/S ratio, liver to spleen ratio; Skin to ROI, the distance from skin surface to the center of ROI for measuring liver (spleen) UDFF.

Table 2: Area under ROC of UDFF and UDFF L/S ratio to determine ≥ mild hepatic steatosis

Parameter	Best Cutoff value	AUC	Sensitivity	Specificity	PPV	NPV
Liver UDFF	7.20%	0.913	90.91%	91.67%	96.77%	78.57%
UDFF L/S Ratio	2.27	0.985	96.97%	100%	100%	92.31%

Note: AUC, area under receiver operating characteristic curve; L/S ratio, liver to spleen ratio; NPV, negative predictive value; PPV, positive predictive value; UDFF, ultrasound derived fat fraction.

5. Discussion

In this preliminary study, we observed significant differences in liver MRI-PDFF, liver UDFF, and UDFF L/S ratio between participants with and without hepatic steatosis. However, the difference in spleen MRI-PDFF or UDFF between participants with and without hepatic steatosis was not significant. Liver UDFF and UDFF L/S ratio closely correlated with liver MRI-PDFF and have high sensitivity and specificity in determining ≥ mild hepatic steatosis.

The study results strengthen the agreement that UDFF is a useful tool in diagnosing mild to severe hepatic steatosis (MRI-PDFF ≥ 5%). Previous research has demonstrated that single parameter of AC, BSC, and combining parameters of AC with BSC (UDFF) of the liver strongly correlate with liver MRI-PDFF and/or biopsy histology in the determination of NAFLD and nonalcoholic steatohepatitis (NASH) [14, 15, 19].

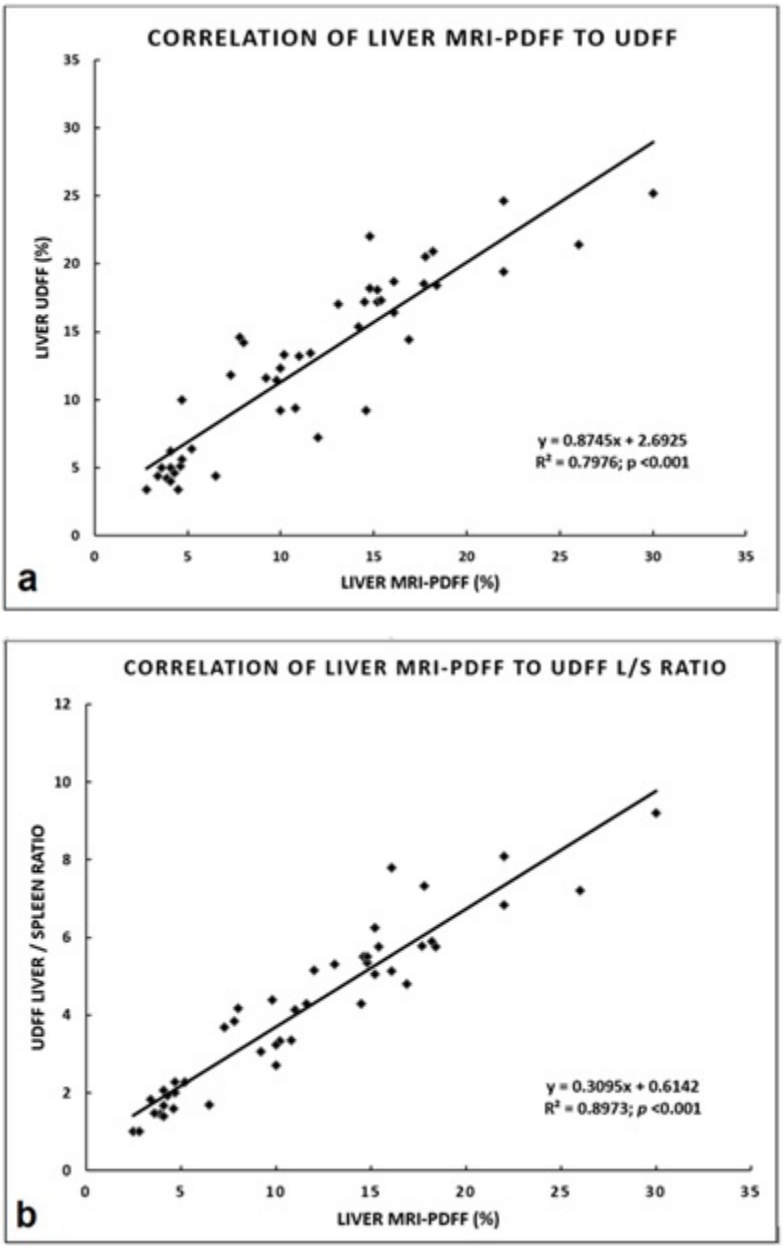


Figure 3a-b: Scatter plots show close positive correlation of liver MRI-PDFF to liver ultrasound derived fat fraction (UDFF) (R^2 =0.80, 3a) and UDFF liver to spleen ratio (R^2 =0.90, 3b).

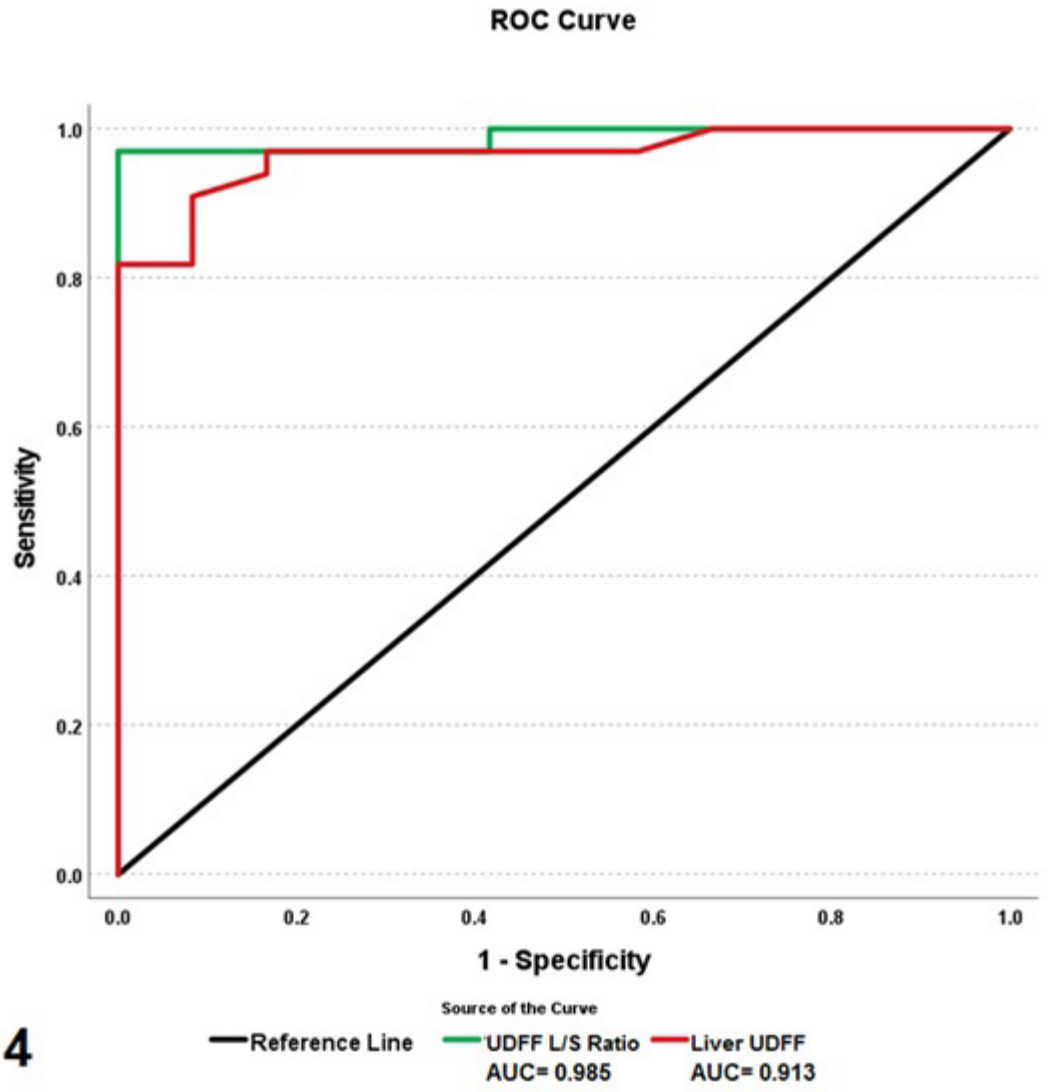


Figure 4: Area under receiver operating characteristic curves (AUC) of ultrasound derived fat fraction (UDFF) and UDFF liver to spleen ration (UDFF L/S ratio) in determining ≥ mild hepatic steatosis is 0.913 and 0.985, respectively.

An important note in the study is that the UDFF L/S ratio strongly correlated with the MRI-PDFF of the liver, despite the number of enrolled participants being small. The correlation between UDFF L/S ratio and liver MRI-PDFF was higher than the correlation between liver UDFF and liver MRI-PDFF (R^2 =0.90 vs R^2 =0.80). In addition, the study demonstrated that there was no significant difference in the spleen fat content assessed by MRI-PDFF and UDFF between participants with and without hepatic steatosis. In other words, the value of spleen UDFF is independent of an increase of UDFF in steatotic livers. This makes the spleen a potential target for the normalization of the liver attenuation coefficient and backscatter coefficient in screening for NAFLD. Although we did not find a research report of spleen UDFF measurement during our literature review, our results agree with the known physiology of the liver and spleen. The liver is involved in the storage and processing of many energy metabolites, including glucose and fat. Once saturated with glycogen, the liver begins to store excess glucose as fatty acids that can be stored within the liver and adipose tissue. The liver assembles fatty acids and glycerol into triglycerides, which are packaged with very low density lipoprotein particles for secretion from hepatocytes into the bloodstream. Therefore, the liver is involved in the storage of excess energy in the form of fat, such as is seen in metabolic syndrome [20]. It is not surprising that both MRI-PDFF and UDFF identified a significant increase in fat content in the livers with steatosis. The function of the spleen is different from that of the liver. It is primarily involved in hematopoietic and immunologic functions. It filters aging erythrocytes and clear encapsulated organisms. In the red pulp of the spleen, pathogens, cellular debris, as well as aging erythrocytes, are efficiently removed from the blood by macrophages. The spleen's white pulp is a highly organized lymphoid region where adaptive immune responses can be initiated. The spleen mounts complex adaptive immune responses, as well as effectively clears pathogens from the blood [21]. The spleen is impacted by sickle cell disease and lymphoproliferative diseases. Unlike the liver, the spleen is not involved in the metabolism and storage of fatty acids. Therefore, it makes the sense to note that the spleen UDFF and MRI-PDFF were

independent from the development of hepatic steatosis in the study. However, further studies must be carried out to test the diagnostic performance of the UDFF L/S ratio as an additional surrogate to single UDFF biomarker in assessing hepatic steatosis.

Historically, a liver-to-kidney ratio obtained by conventional B-mode ultrasound has been used in attempts to determine the presence of hepatic steatosis [22]. There are limitations to using the echogenicity of the kidney cortex as the reference to normalize that of the liver parenchyma. First, the kidney cortex is anisotropic, which can cause an artifact of ultrasound depending on the orientation between the sound beam and cortical tissue [23]. The echogenicity of the kidney cortex varies between longitudinal and transverse sections of the kidney, which may lead estimation error. Second, chronic kidney disease has a prevalence of 8-16% in the general population [24]. Renal parenchymal disease is also common among patients with NAFLD [25]. Interstitial fibrosis in kidney disease alters the tissue echogenicity making renal parenchyma a poor reference to determine the echogenicity of the liver in these patients [26]. Third, anatomic variation of the kidney (horseshoe kidney, polycystic kidney, ectopic kidney) may also invalidate the use of the kidney cortex to normalize echogenicity of liver parenchyma on B-mode ultrasound image [27].

Quantitative AC and BSC biomarkers of the spleen may be of interest in determining the severity of disease processes such as cirrhosis and associated complications such as portal hypertension in individuals with NAFLD. Further investigation may also explore clinical utility of spleen UDFF for assessing other pathologies of the spleen for instance, sickle cell disease, in addition to NAFLD.

There are some limitations in this study. First, the sample size was small. The feasibility of UDFF in grading the severity of hepatic steatosis and in evaluating effects of treatment on liver fat content was not assessed in the study. Second, no histologic sampling of the liver and spleen was available to correlate liver fat content with the UDFF findings. Rather, we relied solely on the correlation of UDFF to MRI-PDFF findings with recognition that MRI-PDFF has been proven reliable in the assessment of fat content in the liver [28]. Third, the study focused on UDFF measurements in participants with and without hepatic steatosis. The effect of liver fibrosis on UDFF value was not analyzed in the study due to small sample size. Fourth, all ultrasound and MRI parameters were measured in adult population. The values of UDFF in the liver and spleen in children need further investigation. Fifth, a senior operator performed all scans. Inter- and intra-observer reliability was not tested in this study. However, good intra-observer repeatability and inter-observer reproducibility in junior and senior operators have been demonstrated previously [16]. Sixth, the diagnostic performance (threshold, sensitivity, specificity) of liver UDFF or UDFF L/S ratio for grading hepatic steatosis was not tested due to the small number of participants recruited in the study. Finally, UDFF is the combination of AC and BSC designed by the manufacturer. Proprietary formulas utilized in calculation of UDFF in the study have not been disclosed to the public by the equipment and software manufacturer, disallowing presentation of that aspect in this report. Therefore, the operator was unable to separate AC or BSC from UDFF measurement. However, spleen AC independent from the development of hepatic steatosis measured using different ultrasound scanner was reported [29].

In conclusion, liver UDFF and the UDFF L/S ratio closely correlate to liver MRI-PDFF and have high sensitivity and specificity in quantifying fat content in the liver. Spleen UDFF is independent from the development of hepatic steatosis in adults.

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