

## Assessment of hepatic fat content in using quantitative ultrasound measurement of hepatic/renal ratio and hepatic echo-intensity attenuation rate

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### Abstract

**Aims:** This study aims to evaluate and validate a simple quantitative ultrasound (US) method for determining the hepatic fat content (HFC) based on the combination of quantitative US hepatic/renal ratio (US-HRR) and quantitative US hepatic echo-intensity attenuation rate (US-HAR) as compared with [<sup>1</sup>H]-magnetic resonance spectroscopy (<sup>1</sup>H-MRS). **Material and methods:** There were a total of 242 subjects recruited in the present study. All subjects were examined for HFC by quantitative US and <sup>1</sup>H-MRS methods. The QUS-HRR and QUS-HAR were calculated from ordinary ultrasound images of liver and kidney with a triple modality 3D abdominal phantom using the Image J software. **Results:** The results found that US-HRR and US-HAR correlated with <sup>1</sup>H-MRS HFC (US-HRR:  $r=0.946$ ,  $p<0.001$ ; US-HAR:  $r=0.936$ ,  $p<0.001$ ). The equation for HFC prediction by using quantitative US was:  $HFC (\%) = 28.965 \times US-HRR + 218.045 \times US-HAR - 8.892$ . Subgroup analysis in study subjects with body mass index (BMI)  $\geq 28$  showed that quantitative US HFC was associated with <sup>1</sup>H-MRS HFC ( $R^2=0.953$ ,  $p<0.001$ ). Receiver operating characteristic (ROC) analysis observed that the cut-off value of fatty liver diagnosis was 6.71% in using the quantitative US model; the sensitivity and specificity for fatty liver diagnosis were 94.15% and 96.30%, respectively. Variability analysis indicated that there was a relative high degree of consistency in the measurement of HFC with different operators or ultrasonic apparatus. **Conclusions:** Quantitative US measurement could be regarded as a simple, sensitive tool to accurately assess HFC. It provides a valid alternative to <sup>1</sup>H-MRS as an easy, non-invasive option for the precise estimation of HFC in clinical practice.

**Keywords:** type 2 Diabetes; hepatic fat content; ultrasound hepatic/renal ratio; ultrasound hepatic echo-intensity attenuation rate; magnetic resonance spectroscopy

### Introduction

Non-alcoholic fatty liver disease (NAFLD) has become a major public health issue with a worldwide epidemic [1]; the prevalence of NAFLD has been increasing in recent years (30% in Europe and America and about 15% in Asia). In China, it has been estimated that the prevalence rate of NAFLD is 6% to 27%, and the incidence rate of NAFLD is about 3.4% to 9.1% every year [2-4]. The close relationship between NAFLD and diabe-

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tes has been well demonstrated, where NAFLD associated with the increased risk for insulin resistance, and there was also a higher risk of diabetes prevalence in patients with NAFLD [5,6]. In addition, several studies have revealed that the presence of NAFLD was also associated with central obesity, metabolic disorders and cardiovascular diseases [7-9]. In this context, precise evaluation of hepatic liver content (HFC) is not only of great importance for the early diagnosis of NAFLD, but also is useful in predicting the risk for future occurrence of diabetes, metabolic disorders and cardiovascular diseases.

Currently, non-targeted percutaneous liver biopsy with direct histological visualization is the current gold standard to diagnose NAFLD, but its widespread use is limited by cost, sampling error, and procedure-related morbidity and mortality. In the past few years, a number of studies have reported a high sensitivity of the noninvasive technique of [<sup>1</sup>H] magnetic resonance spectroscopy (<sup>1</sup>H-MRS) in the assessment of HFC but routine use of <sup>1</sup>H-MRS is limited by its cost and availability [10-13].

Ultrasound (US) is the most widely available modality for the initial evaluation of hepatic steatosis and diagnosing NAFLD, but this method could be affected by subjective factors, thus causing an inaccurate result. Qualitative US is able to infer the presence and severity according to qualitative sonographic features of the US hepatic/renal ratio (US-HRR) or the US hepatic echo-intensity attenuation rate (US-HAR) [14,15]. Previous literature have found a significant correlation of US-HRR with histologic steatosis and the hepatic/renal sonographic index in patients with chronic liver diseases [16]; in addition, it has also been revealed that US-HRR and US-HAR associated with the degree of liver steatosis and the development of NAFLD [17-19]. However, given the several shortcomings of previous studies, such as the small sample size, ethnic variations and differed study subjects, the development of an easy, simple and accurate quantitative US measurement for detecting HFC is beneficial for the identification of asymptomatic high-risk NAFLD populations and the evaluation of appropriate therapy response or disease progression in NAFLD patients.

In the present study, we established a quantitative US method to provide a more precise estimation on HFC with a triple modality 3D abdominal phantom combined with quantitative US-HRR and quantitative US-HAR as compared with <sup>1</sup>H-MRS.

## Material and methods

### Study subjects

Two hundred and forty-two study subjects [141 consecutively newly diagnosed type 2 diabetes mellitus

(NT2DM) patients, 48 prediabetes mellitus (PDM) subjects and 53 normal controls (NC)] were recruited from the Department of Endocrinology or the medical examination center at the Second People's Hospital of Hefei, when they first visited the DM clinic. The diagnosis of T2DM or PDM was verified according to the American Diabetes Association diagnostic criteria (2018) [20]. NC subjects, without any liver or metabolic diseases, were enrolled from the medical examination center. Patients with other causes of liver disease (viral hepatitis, autoimmune hepatitis, Wilson's disease, hemochromatosis, drug-induced hepatitis, or others) were excluded. Anthropometric measurement and routine laboratory results were obtained from hospital medical records.

All subjects completed the following tests when first entering the study: fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST),  $\gamma$ -glutamyltransferase (GGT), lactate dehydrogenase (LDH), total bilirubin (TBIL), indirect bilirubin (IBIL), direct bilirubin (DBIL), creatinine, uric acid (UA), apolipoprotein (Apo)A1 and ApoB.

All the blood biochemical indices were determined by using a Hitachi 7600 autoanalyzer (Hitachi Ltd., Tokyo, Japan) or immunoturbidimetric assay (Roche/Cobas Integra Tina Quant, Roche Diagnostics).

### Standard protocol approvals and patient consents

This study was approved by the Ethical Committee of the Second People's Hospital of Hefei (Hefei, Anhui, China). All the study subjects provided informed consent to participate in this study.

The study was conducted according to the principles of the Declaration of Helsinki (1964) and its amendments.

### HFC detection by <sup>1</sup>H-MRS

<sup>1</sup>H-MRS was used to detect the HFC of all subjects. The right lobe of the liver was located when patients were lying in the supine position [21]. Areas under the water peak and fat peak were recorded. Liver fat content was calculated as [liver fat content (%) = area under the fat peak  $\times$  100/(area under the fat peak + area under the water peak)]. Liver fat content  $\geq$ 5.56% was defined as fatty liver [13].

### US-HRR and US-HAR analysis

US examinations were implemented to measure the HFC of all study subjects at the same day with the <sup>1</sup>H-MRS detection. Ultrasonic images were analyzed by Image J software (Image J2x, National Institutes of Health, USA; <https://imagej.nih.gov/ij/>). Average gray-scale intensities

were determined in the regions of interest (ROIs) of liver (15.11×15.11 mm) and renal cortex (5.14×5.14 mm). The hepatic/renal ratio was calculated according to the equation: hepatic/renal ratio = mean gray-scale intensity of the liver/mean grayscale intensity of the renal cortex (fig 1a). Two liver ROIs samples were selected at a depth of 4-6 cm from the near-field of the same beam. The distance between the two ROIs were measured (fig 1b). The hepatic echo-intensity attenuation rate was calculated according to the equation: hepatic echo-intensity attenuation rate =  $(\ln A_n - \ln A_f) / (\Delta d \times f)$  [22]; where  $A_n$  and  $A_f$  represent the mean echo intensity of the near-field and far-field ROIs, respectively;  $\Delta d$  is the line distance between the two ROIs and  $f$  is the ultrasonic transducer frequency.

**Adjustment for quantitative US parameters**

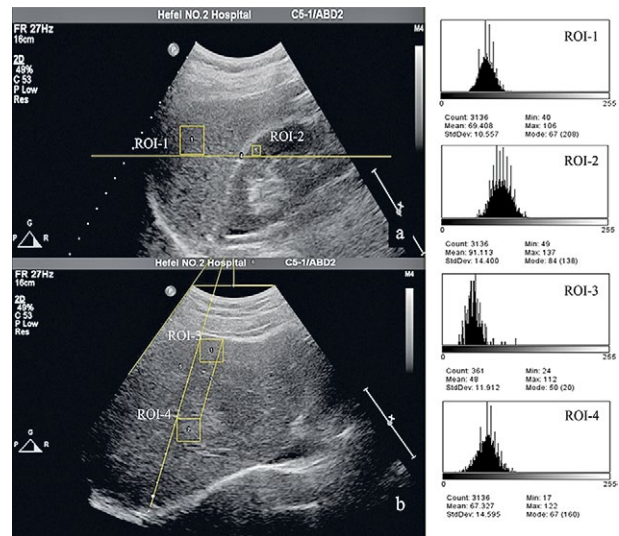
To correct the US-HRR and US-HAR, we applied a triple modality 3D abdominal phantom to adjust the normal distribution of abdominal organs of human body [23-25]. The triple modality 3D abdominal phantom stimulated images were analyzed, then the quantitative US-HRR and quantitative US-HAR were calculated according to the equation: quantitative US-HRR = US-HRR of study subjects / HRR of 3D model; quantitative US-HAR = US-HAR of study subjects - HAR of 3D model.

**Variability analysis**

To evaluate the consistency of quantitative US-HRR and quantitative US-HAR among different operators in using the same ultrasonic device, the repeated measures on quantitative US-HRR and quantitative US-HAR were performed in 100 study subjects by two independent US specialists who had over 10 years of experience (Gui-Ping Zhang and Xiao Yang). In addition, to compare the variability of different ultrasonic devices on the influence of quantitative US-HRR and quantitative US-HAR, an experienced sonographer independently measured the quantitative US-HRR and quantitative US-HAR among 100 study subjects in using two different types of medical ultrasonic devices, respectively (IE33 [Philip, Germany], transducers [C5-1, Philip, Germany] and [GE Logiq P7, USA], transducers [C1-5 D, GE, USA]).

**Statistical analysis**

Normal distribution data were represented as a mean ± standard deviation; if data was not in the normal distribution, the median and interquartile range was used. One-way ANOVA or nonparametric test (Kruskal-Wallis test) was utilized for intergroup comparisons. Linear regression analyses were used to detect the associations of quantitative US-HRR/US-HAR HFC with <sup>1</sup>H-MRS HFC. Spearman correlation analysis was performed to investigate the correlation of the quantitative US determined HFC with several clinical/laboratory parameters. Variability analysis was performed by calculating intra-



**Fig 1.** Ultrasonic liver image with HRR and HAR: a) Ultrasonic liver image with HRR. ROI-1 and ROI-2 stands for the echo gray histograms of the liver and kidney cortex ROIs; b) Ultrasonic liver image with HAR. ROI-3 and ROI-4 stands for the near-field and far-field echo gray histograms of the liver. HAR: hepatic echo-intensity attenuation rate; HRR: hepatic/renal ratio; ROI: Region of interest.

class correlation coefficients and depicted Bland-Altman plots [26]. Receiver operating characteristic (ROC) analysis was constructed to evaluate the sensitivity and specificity of quantitative US determined HFC for the diagnosis of NAFLD. Data analysis was performed in using the SPSS23.0 statistical software (SPSS Inc., Chicago, IL, USA) and MedCalc, version 11.4.2.0 (Mariakerke, Belgium). Two tailed  $p < 0.05$  were considered to be statistically significant.

**Results**

**Characteristics of the study population**

There were 188 subjects diagnosed as NAFLD (77.68%, 188/242) based on HFC  $\geq 5.56\%$ . Given the differences of <sup>1</sup>H-MRS HFC, we divided all study subjects into five groups (HFC < 5% (n=48), HFC: 2.96±1.12%; 5% ≤ HFC < 10% (n=65), HFC: 7.39±1.33%; 10% ≤ HFC < 15% (n=50), HFC: 13.18±1.42%; 15% ≤ HFC < 20% (n=55), HFC: 17.03±1.33%; 20% ≤ HFC (n=24), HFC: 24.60±2.86%). There were significant differences in body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, 2hPG, BUN, UA, TBIL, IBIL, ALT, GGT, TG, TC, HDL-C, LDL-C, VLDL-C, ApoA1, <sup>1</sup>H-MRS HFC, quantitative US-HRR and quantitative US-HAR among those five groups (all  $p < 0.05$ ). However, we did not observe any

significant difference regarding gender distribution, age, Cr, DBIL, AST, ALP, LDH and ApoB in those five groups (all  $p > 0.05$ ). In addition, we found that the increase of quantitative US-HRR and quantitative US-HAR was consistent with the gradual increase of  $^1\text{H-MRSHFC}$  (Table I).

#### **Correlation between quantitative US parameters and $^1\text{H-MRS HFC}$**

Correlation analysis revealed that quantitative US-HRR and quantitative US-HAR were highly correlated with  $^1\text{H-MRS HFC}$ , respectively ( $r=0.946$ ,  $p < 0.001$ ;  $r=0.936$ ,  $p < 0.001$ ) (fig 2).

#### **Defining the quantitative US model for HFC estimation**

Linear regression analysis was applied to evaluate quantitative US parameters for HFC estimation.  $^1\text{H-MRS HFC}$  was set as a dependent variable, and quantitative US-HRR was included in the regression equation as independent variable. The results indicated that quantitative US-HRR was the strong predictor for HFC (corrected  $R^2=0.895$ ,  $p < 0.001$ ). When quantitative US-HAR combined quantitative US-HRR into the regression model, it showed an improved estimation accuracy (Corrected  $R^2=0.935$ ,  $p < 0.001$ ). The equation for HFC prediction by using quantitative US was:  $\text{HFC (\%)} = 28.965 \times \text{quantitative US-HRR} + 218.045 \times \text{quantitative US-HAR} - 8.892$  (Table II).

#### **Correlation analysis between quantitative USHFC and clinical/laboratory parameters**

The quantitative US HFC was positively correlated with BMI, WHR, SBP, DBP, FPG, 2hPG, UA, ALT, GGT, TG, TC, LDL-C and VLDL-C and negatively correlated with LDH, HDL-C and ApoA1 (Table III).

#### **Correlation of $^1\text{H-MRS HFC}$ and quantitative US HFC**

To avoid the potential effect of subcutaneous fat in obese subjects on the echo attenuation, we performed a correlation analysis of  $^1\text{H-MRS HFC}$  and quantitative US

HFC in study subgroup with  $\text{BMI} \geq 28$ . The results indicated that quantitative USHFC was still associated with  $^1\text{H-MRS HFC}$  ( $R^2 = 0.953$ ,  $p < 0.001$ ) (fig 3).

#### **NAFLD diagnosis by quantitative US and $^1\text{H-MRS}$**

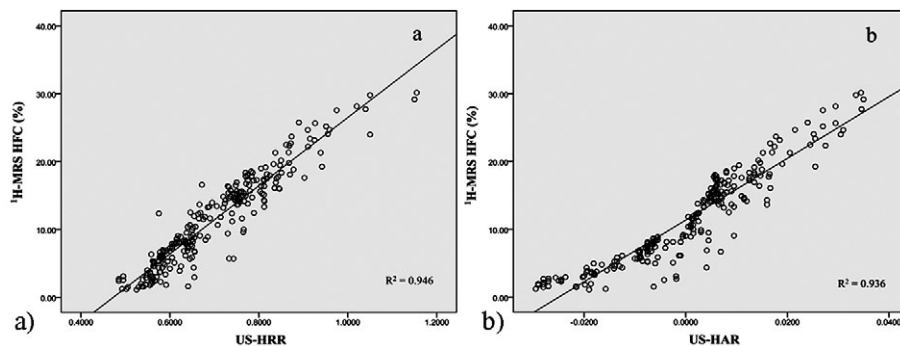
ROC analysis revealed that the optimum point of fatty liver diagnosis was 6.71% in using the quantitative US model. Based on the  $^1\text{H-MRS HFC}$ , all study subjects were divided into the NAFLD group ( $\text{HFC} \geq 5.56\%$ ) and non-NAFLD group. When  $^1\text{H-MRS}$  was set as the gold standard for diagnosing NAFLD by the quantitative US model, the sensitivity and specificity for NAFLD diagnosis were 94.15% and 96.30%, with the area under curves (AUC) of 0.987 (95%CI: 0.963-0.997). Furthermore, a subgroup analysis was also implemented when  $^1\text{H-MRS HFC} < 11.12\%$ , the sensitivity and specificity for quantitative US model were 95.31% and 90.74%, with the AUC of 0.963 (95%CI: 0.912-0.989).

#### **Variability analysis**

Intraclass correlation coefficient was calculated to test the consistency of quantitative US HFC in different operators or medical ultrasonic devices; the results implied that there were relatively high degrees of consistencies between different operators or ultrasonic devices (Table IV, fig 4)

#### **Bland-Altman analysis for evaluation of the quantitative US HFC**

To avoid the omission of subjects with light fatty liver, the cut-off value was set as 2-fold of diagnostic standard ( $\text{HFC} \geq 5.56\%$  defined as fatty liver). First, 118 study subjects with  $^1\text{H-MRS HFC} < 11.12\%$  were included in the Bland Altman analysis. The results found that there was no significant bias for 118 study subjects considered; six subjects (6/118) showed an overestimated HFC, and one subject (1/118) had an underestimated HFC. Moreover, when the Bland Altman analysis was also performed in 242 study subjects, the results observed that while twelve subjects (12/242) had a higher HFC, only four subjects (4/242) reported a lower HFC.



**Fig 2.** Correlation analysis between  $^1\text{H-MRS HFC}$  and US-HRR (a), and US-HAR (b). US-HAR: ultrasound hepatic echo-intensity attenuation rate; US-HRR: ultrasound hepatic/renal ratio;  $^1\text{H-MRS}$ : [ $^1\text{H}$ ]-magnetic resonance spectroscopy; HFC: hepatic fat content



Table I. Clinical characteristic of different HFC groups

| Parameters                                    | HFC<5%<br>(n=48)        | 5%≤HFC<10%<br>(n=65)    | 10%≤HFC<15%<br>(n=50) | 15%≤HFC<20%<br>(n=55) | 20%≤HFC<br>(n=24)    | p value |
|---|-------------------------|-------------------------|-----------------------|-----------------------|----------------------|---------|
| Gender (female/male)                          | 29/19                   | 29/36                   | 22/28                 | 24/31                 | 13/11                | 0.360   |
| Age (years)                                   | 46.3±9.2                | 49.6±10.1               | 52.3±10.8             | 50.9±11.1             | 49.3±9.6             | 0.074   |
| BMI (kg/m <sup>2</sup> )                      | 23.60±2.78              | 25.17±2.00              | 26.68±2.01            | 26.92±3.09            | 27.25±2.28           | <0.001  |
| WHR   | 0.87±0.06               | 0.91±0.06               | 0.97±0.10             | 0.98±0.09             | 1.01±0.12            | <0.001  |
| SBP (mmHg)                                    | 125±13                  | 131±16                  | 134±15                | 139±17                | 137±14               | <0.001  |
| DBP (mmHg)                                    | 77±9                    | 81±11                   | 82±11                 | 87±9                  | 84±11                | <0.001  |
| FPG (mmol/L)                                  | 5.26±1.07               | 5.94±1.19               | 6.96±1.43             | 7.21±1.20             | 7.82±0.83            | <0.001  |
| 2hPG (mmol/L)                                 | 6.49 (5.71, 9.49)       | 9.29 (7.40, 15.35)      | 15.70 (10.88, 17.03)  | 16.13 (12.61, 17.68)  | 17.03 (15.73, 17.88) | <0.001  |
| BUN (mmol/L)                                  | 4.52±1.29               | 5.13±1.48               | 5.37±1.60             | 5.25±1.38             | 4.67±1.07            | 0.016   |
| Cr (umol/L)                                   | 60.80±13.60             | 59.76±13.18             | 59.38±16.23           | 59.99±14.87           | 54.71±13.06          | 0.531   |
| UA (umol/L)                                   | 292.00±66.00            | 320.82±64.05            | 345.08±70.15          | 331.49±53.51          | 366.13±65.07         | <0.001  |
| TBIL (umol/L)                                 | 13.80 (11.80, 16.19)    | 17.00 (11.60, 22.70)    | 16.95 (13.43, 22.78)  | 15.70 (12.70, 20.60)  | 14.05 (11.05, 17.83) | 0.038   |
| DBIL (umol/L)                                 | 4.01±1.29               | 4.99±2.60               | 4.46±1.78             | 4.39±2.16             | 3.82±1.30            | 0.056   |
| IBIL (umol/L)                                 | 9.90 (8.10, 11.98)      | 12.50 (8.85, 17.65)     | 12.80 (10.28, 15.93)  | 11.20 (9.00, 15.90)   | 10.20 (7.93, 13.15)  | 0.018   |
| ALT (U/L)                                     | 21.50 (13.25, 33.50)    | 32.00 (28.00, 36.00)    | 33.00 (31.00, 40.00)  | 35.00 (30.00, 38.00)  | 35.00 (32.00, 38.75) | <0.001  |
| AST (U/L)                                     | 19.00 (16.00, 23.75)    | 21.00 (17.00, 26.00)    | 22.00 (16.00, 27.00)  | 21.00 (17.00, 33.00)  | 19.00 (14.50, 29.50) | 0.256   |
| GGT (U/L)                                     | 23.00 (17.00, 38.75)    | 33.00 (22.00, 63.00)    | 33.00 (19.75, 48.75)  | 32.00 (26.00, 54.00)  | 37.00 (26.00, 57.50) | 0.007   |
| ALP (U/L)                                     | 69.56±22.08             | 76.80±22.03             | 77.12±19.92           | 75.60±20.59           | 76.75±20.09          | 0.362   |
| LDH (U/L)                                     | 176.08±33.36            | 178.75±36.78            | 175.76±34.98          | 171.09±36.12          | 164.54±31.21         | 0.469   |
| TG (mmol/L)                                   | 1.62±0.73               | 2.02±0.78               | 2.28±0.68             | 2.30±0.71             | 2.43±0.51            | <0.001  |
| TC (mmol/L)                                   | 4.45±0.70               | 4.81±0.49               | 5.21±0.42             | 5.24±0.44             | 5.32±0.31            | <0.001  |
| HDL-C (mmol/L)                                | 1.55±0.38               | 1.40±0.30               | 1.43±0.32             | 1.42±0.30             | 1.25±0.18            | 0.003   |
| LDL-C (mmol/L)                                | 2.34 (2.04, 3.01)       | 2.89 (2.21, 3.31)       | 3.16 (2.79, 3.42)     | 3.18 (2.98, 3.42)     | 3.17 (2.95, 3.40)    | <0.001  |
| VLDL-C (mmol/L)                               | 0.25 (0.18, 0.37)       | 0.33 (0.23, 0.54)       | 0.36 (0.26, 0.62)     | 0.34 (0.24, 0.53)     | 0.45 (0.37, 0.71)    | 0.002   |
| ApoB (g/L)                                    | 0.94 (0.81, 1.04)       | 0.90 (0.72, 1.06)       | 0.92 (0.74, 1.07)     | 0.86 (0.75, 1.02)     | 0.85 (0.66, 0.93)    | 0.493   |
| ApoA1 (g/L)                                   | 1.25±0.26               | 1.14±0.20               | 1.16±0.21             | 1.13±0.18             | 1.08±0.18            | 0.009   |
| HFC%  | 2.96±1.12               | 7.39±1.33               | 13.18±1.42            | 17.03±1.33            | 24.60±2.86           | <0.001  |
| US-HRR  | 0.56±0.04               | 0.63±0.05               | 0.72±0.05             | 0.8±0.05              | 0.95±0.09            | <0.001  |
| US-HAR (MHz <sup>-1</sup> *cm <sup>-1</sup> ) | -0.019 (-0.025, -0.016) | -0.007 (-0.008, -0.001) | 0.004 (0.002, 0.007)  | 0.008 (0.006, 0.013)  | 0.025 (0.018, 0.03)  | <0.001  |

ApoA1: Apolipoprotein-A1; ApoB: Apolipoprotein-B; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine transaminase; BMI: body mass index; Cr: creatine; DBIL: direct bilirubin; DBP: diastolic blood pressure; FBG: fasting blood glucose; GGT:  $\gamma$ -glutamyltransferase; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA- $\beta$ : home beta cell function index; HDL-C: high-density lipoprotein cholesterol; IBIL: indirect bilirubin; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides; TBIL: total bilirubin; UA: uric acid; VLDL-C: very low-density lipoprotein cholesterol; WHR: waist-to-hip ratio; HFC: hepatic fat content; US-HRR: ultrasound hepatic/renal ratio; US-HAR: ultrasound hepatic echo-intensity attenuation rate

Table II. Quantified US parameters predicts HFC model

|     | Model | US-HRR |         | US-HAR  |         | Constant | Corrected R <sup>2</sup> |
|-----|-------|--------|---------|---------|---------|----------|--------------------------|
|     |       | β      | p       | β       | p       |          |                          |
| HFC | 1     | 50.240 | < 0.001 | -       | -       | -23.752  | 0.895                    |
|     | 2     | 28.965 | < 0.001 | 218.045 | < 0.001 | -8.892   | 0.935                    |

Model 1: US -HRR to estimate 1H-MRS HFC. Model 2: US-HRR and US-HAR to estimate 1H-MRS HFC. US: ultrasound; US-HRR: ultrasound hepatic/renal ratio; US-HAR: ultrasound hepatic echo-intensity attenuation rate

Table III. Correlation of quantitative US quantified HFC with clinical characteristic.

| Parameters | Quantitative US quantified HFC |              |
|------------|--------------------------------|--------------|
|            | r                              | P            |
| Age        | 0.096                          | 0.137        |
| BMI        | 0.500                          | <b>0.000</b> |
| WHR        | 0.561                          | <b>0.000</b> |
| SBP        | 0.287                          | <b>0.000</b> |
| DBP        | 0.266                          | <b>0.000</b> |
| FBG        | 0.574                          | <b>0.000</b> |
| 2hPG       | 0.553                          | <b>0.000</b> |
| BUN        | 0.107                          | 0.096        |
| Cr         | -0.046                         | 0.477        |
| UA         | 0.298                          | <b>0.000</b> |
| TBIL       | 0.010                          | 0.876        |
| DBIL       | -0.047                         | 0.464        |
| IBIL       | 0.019                          | 0.770        |
| ALT        | 0.341                          | <b>0.000</b> |
| AST        | 0.044                          | 0.492        |
| GGT        | 0.174                          | <b>0.007</b> |
| ALP        | 0.107                          | 0.097        |
| LDH        | -0.127                         | <b>0.048</b> |
| TG         | 0.311                          | <b>0.000</b> |
| TC         | 0.464                          | <b>0.000</b> |
| HDL-C      | -0.183                         | <b>0.004</b> |
| LDL-C      | 0.394                          | <b>0.000</b> |
| VLDL-C     | 0.230                          | <b>0.000</b> |
| ApoB       | -0.092                         | 0.155        |
| ApoA1      | -0.166                         | <b>0.010</b> |

ApoA1: Apolipoprotein-A1; ApoB: Apolipoprotein-B; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine transaminase; BMI: body mass index; Cr: creatine; DBIL: direct bilirubin; DBP: diastolic blood pressure; FBG: fasting blood glucose; GGT: γ-glutamyltransferase; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homa beta cell function index; HDL-C: high-density lipoprotein cholesterol; IBIL: indirect bilirubin; LDH: lactate dehydrogenase; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides; TBIL: total bilirubin; UA: uric acid; VLDL-C: very low-density lipoprotein cholesterol; WHR: waist-to-hip ratio; HFC: hepatic fat content; US: ultrasound

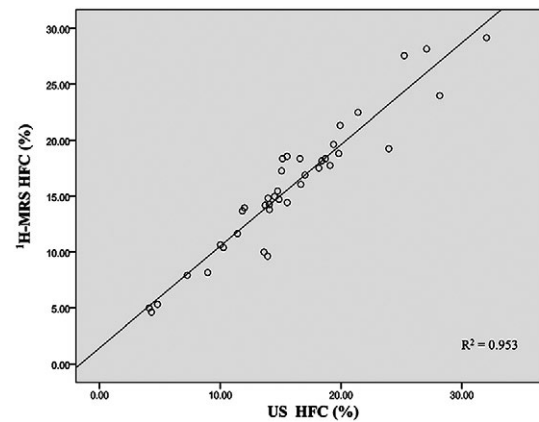


Fig 3. Correlation analysis between 1H-MRS HFC and US-HRR (a), and US-HAR (b). US-HAR: ultrasound hepatic echo-intensity attenuation rate; US-HRR: ultrasound hepatic/renal ratio; 1H-MRS: [1H]-magnetic resonance spectroscopy; HFC: hepatic fat content

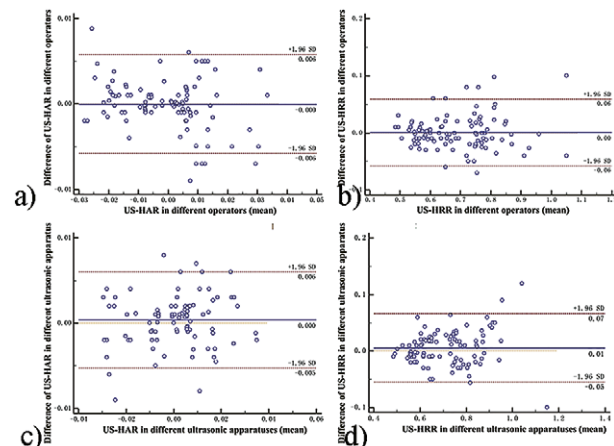


Fig 4. Bland–Altman analysis for intra-session repeatability for different operators in HAR (a), HRR (b); for different ultrasonic apparatuses in HAR (c), HRR (d). HAR: hepatic echo-intensity attenuation rate; HRR: hepatic/renal ratio

Table IV. Intraclass correlation coefficient of quantitative US parameters by different operators or apparatuses.

| Parameters            | US parameters | Intraclass correlation coefficient | p      | 95% CI |       |
|-----------------------|---------------|------------------------------------|--------|--------|-------|
| Different operators   | US-HRR        | 0.970                              | <0.001 | 0.956  | 0.980 |
|                       | US-HAR        | 0.978                              | <0.001 | 0.967  | 0.985 |
| Different apparatuses | US-HRR        | 0.972                              | <0.001 | 0.958  | 0.981 |
|                       | US-HAR        | 0.981                              | <0.001 | 0.972  | 0.987 |

US: ultrasound; US-HRR: ultrasound hepatic/renal ratio; US-HAR: ultrasound hepatic echo-intensity attenuation rate

## Discussion

Over the past decade, emerging evidence has highlighted the relationship of an excessive hepatic fat accumulation with several disease risks for future occurrence of diabetes, metabolic disorders and cardiovascular diseases [7-9]. Therefore, the early detection of HFC is of great importance in providing information about the liver and in the assessment and prevention of cardio-metabolic risks.

The common recognized gold standard for the assessment of fatty liver is liver biopsy, but this technique is invasive and suffers from sampling problems resulting in diagnostic errors; moreover, the result is also strongly influenced by the subjectivity and experience of the pathologist [27,28]. As an alternative, non-invasive approaches can be used to quantify liver steatosis,  $^1\text{H-MRS}$  has been recognized as a sensitive, accurate and quantitative evaluation of HFC using non-ionizing radiation, as compared to liver biopsy,  $^1\text{H-MRS}$  showed a comparative sensitivity (100%) and specificity (97%) in the diagnosis of liver steatosis [13]. However, the use of this approach in clinical practice is limited because of the time required for examination, high equipment requirements and high expense. In addition, both the acquisition and analysis of the collected spectra require expertise and specialized software which further restricts the availability of this technique.

In the present study, we have investigated the association of quantitative US HFC with  $^1\text{H-MRS}$  HFC by enrolling a total of 242 subjects, and have also constructed the equation of the quantitative US model for HFC estimation. In addition, the variability analysis and Bland-Altman analysis were also utilized to assess the consistency of quantitative US HFC in different operators or medical ultrasonic devices. The present study revealed that the quantitative US-HRR and quantitative US-HAR were highly correlated with  $^1\text{H-MRS}$  HFC. In addition, when using quantitative US-HRR and quantitative US-HAR to predict the  $^1\text{H-MRS}$  HFC; the results of MLR analysis supported a relatively increased accuracy in using a combination of quantitative US-HRR and quantitative US-HAR for the prediction of HFC. These indicated that the use of the quantitative US model could represent a valid alternative to  $^1\text{H-MRS}$  imaging. Considering overweight and obese subjects, quantitative US HFC was still associated with  $^1\text{H-MRS}$  HFC in the subgroup of  $\text{BMI} \geq 28$ . These results suggested that the quantitative US model has a good diagnostic performance even in overweight and obese patients.

We have also compared the performance of quantitative US and  $^1\text{H-MRS}$  in the diagnosis of NAFLD, the ROC

analysis revealing that the quantitative US model exerted a good sensitivity (94.15%) and specificity (96.30%) in the diagnosis of NAFLD (in compared with  $^1\text{H-MRS}$ ), with the AUC of 0.987 (95% CI: 0.963-0.997). The quantitative US model showed a cut-off point of 6.71% in the diagnosis of NAFLD, which turned out to be higher than that of  $^1\text{H-MRS}$  data ( $\text{HFC} \geq 5.56\%$ ); this discrepancy may be due to the imperfectly linear relationship between quantitative US HFC and  $^1\text{H-MRS}$  HFC. Furthermore, when we set the cut-off value as  $^1\text{H-MRS}$  HFC  $< 11.12\%$ , the results revealed that the sensitivity and specificity for quantitative US were 95.31% and 90.74%, with the AUC of 0.963 (95%CI: 0.912-0.989). These facts confirmed that quantitative US is capable of providing a precise and reliable diagnostic value for the assessment of NAFLD and fatty liver degeneration.

Currently, given the features of non-invasive, non-ionizing, inexpensive and widely available in US, several studies have been conducted to use US in the determination of HFC [29-31]. However, the sensitivity and specificity of using US in the quantification of HFC have differed. A meta-analysis, performed by Bohte et al, implied that the use of conventional US in the assessment of NAFLD showed a sensitivity of 73% to 91% as compared to MRS, and they also observed that the conventional US does not accurately predict the presence of NAFLD when HFC is  $< 10\%$ , with the sensitivity of 62.2% to 82.1% [32]. Bedossa et al showed that the conventional US had the sensitivity of 55% in diagnosis of NAFLD when HFC  $< 20\%$  [33]. It has also been reported that the sensitivities and specificities of using conventional US in assessing liver fat ranges from 60% to 94% [34-37]. The different parameter settings among ultrasonic devices, post-processing procedures for US images and ultrasonic operators that may contribute to the varied sensitivities and specificities of using conventional US in assessing HFC, restrict the clinical reliability of using US in the quantification of HFC.

In the present study, we have adopted the triple modality 3D abdominal phantom to adjust the potential errors that may be caused by different ultrasonic operators and devices. We found that quantitative US with a triple modality 3D abdominal phantom adjustment in the assessment of HFC represented a good performance as compared with  $^1\text{H-MRS}$  HFC. The results of the variability analysis also indicated that the quantitative US HFC between two independent operators or two types of ultrasonic devices had a good repeatability. The Bland-Altman analysis did not reveal a significant bias for most of the study subjects in the use of quantitative US as compared with  $^1\text{H-MRS}$ , but it showed that quantitative US had an allowable overestimation in the assessment

of early liver steatosis. These results, along with a previous study implementing automatic measurements on US images [18,19], supported that the assessment of HFC in using the quantitative US model could decrease the variability related to the subjective visual evaluation, the influence of different ultrasonic devices and operators. This suggested that the application of quantitative US is an acceptable technique for the detection of fatty liver and NAFLD.

There are some shortcomings that should be observed in the present study. First, liver biopsy is still the common recognized standard for diagnosing fatty liver. Our study used <sup>1</sup>H-MRS HFC as the standard comparison group rather than histology, thus this may lead to a potential discrepancy in the assessment of HFC and the diagnosis of NAFLD. Second, our study has shown that quantitative US exerted an excellent performance for the detection of NAFLD in subjects with moderate and high HFC, but it was limited in subjects with a low HFC. Furthermore, the present study was a single-hospital based study with a relatively small study sample size. Thus, it could possibly restrict the generalizability of our findings. A further multi-center study with a large scale population is required to confirm our results.

### Conclusions

Overall, our study established and confirmed the application of quantitative US model with a triple modality 3D abdominal phantom for a relatively accurate estimation of HFC through the combination of quantitative US-HRR and quantitative US-HAR, suggesting that quantitative US is capable of being a valid alternative to <sup>1</sup>H-MRS as a non-invasive, reliable option with low costs in the clinical assessment of liver fat and diagnosis of NAFLD.

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**Conflict of interest:** none

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