

Dear Professor Lian-Sheng Ma,
Founder and Chief Executive Officer,

On behalf of co-authors, we thank you very much for considering a revised version of our manuscript "Liver fat accumulation measured by high-speed T2-corrected multi-echo MR spectroscopy can predict the risk of cholelithiasis" (Manuscript ID: 57087), which we wish to be considered for publication in *World Journal of Gastroenterology*. We thank the reviewer for the kind comments and suggestions. We have thoroughly corrected the manuscript based on editor and reviewers' comments. In the revised manuscript, we have added the "article highlights" section at the end of the main text and provided the original figures using PowerPoint. As below, we list the changes, and provide point-to-point responses to the reviewer's comments. The changes we made to the text are highlighted in red.

We do hope that the revised manuscript can be judged as acceptable for publication in *World Journal of Gastroenterology*. If any more responses are considered to be necessary, please let me know.

Looking forward to hearing from you soon.

Best regards,

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Point-to-point Response

Reply to reviewers

Comment 1: In the statistical analysis section, authors should indicate which normality tests have been used to assess the Gaussian distribution of the data since samples size are rather small.

Response: Thank you for your reminding. Since the samples size are rather small in our study, data distributions should be tested for normality. According to your suggestions, we have tested the data for normality with Shapiro-Wilk test in this study. Mean values were compared between cholelithiasis group and control group by either Student's t-test or non-parametric Mann-Whitney U-test. The differences of parameters between cholelithiasis group and control group were compared by using Student's t-test (when data were normally distributed) or non-parametric Mann-Whitney U-test (if the data were not normally distributed).

Revised manuscript (Statistical analysis, page 8, line 15-18): "Data distributions were tested for normality with Shapiro-Wilk test. Mean values were compared between the cholelithiasis group and control group by either Student's t-test (when data were normally distributed) or non-parametric Mann-Whitney U-test (when data were not normally distributed)."

Comment 2: In the Results section: Paragraph Demographic and clinical characteristics of the study population Results of normality test for the various variables should be given.

Response: Thank you for your kind comment. The result of normality test for various variables were as follows: age, waist circumference, serum iron, serum iron saturation, transferrin and hemoglobin were normally distributed; serum alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), total bilirubin (TBIL), alkaline phosphatase (ALP), serum uric acid, serum uric glucose, total cholesterol, triglycerides, serum ferritin, PDFP and R2 were not normally distributed. According to your suggestions, we have re-analyzed the data in this study.

Although there were some changes in the results of statistical analysis, it should be pointed out

that whether there were statistical differences of these parameters between the two groups and the final results are as the same as the previous results. These corrections will not change the statistical differences between the two groups in the original text, nor the conclusion of the original manuscript.

Thanks again for your suggestions to make this article more rigorous. The revised changes were showed in **Table 1**.

Table 1 Demographic, anthropometric, biochemical and MRI parameters in patients with cholelithiasis and healthy controls

Characteristics	Cholelithiasis (n=40)	Control (n=31)	<i>P</i> value
Demographic			
Age (years)	54.8±14.6	50.6±14.3	0.235
Women	42.5%(17/40)	32.3%(10/31)	0.463
Anthropometric			
Waist circumference (cm)	85.3±9.0	81.0±6.9	0.030*
Biochemical			
ALT (U/L)	84.6±115.9	69.5±156.2	^a 0.226
AST (U/L)	61.0±94.9	52.9±75.5	^a 0.330
TBIL (μmol/L)	29.4±56.6	19.5±17.6	^a 0.921
GGT (U/L)	246.3±317.8	103.8±146.4	^a 0.037*
ALP (U/L)	146.3±122.8	125.2±92.1	^a 0.382
Serum uric acid (μmol/L)	332.0±121.0	375.9±107.4	^a 0.190
Serum uric glucose (mmol/L)	5.6±1.7	6.3±3.6	^a 0.606
Total cholesterol (mg/L)	5.2±1.5	5.1±1.2	^a 0.662
Triglycerides (mmol/L)	2.0±1.7	1.2±0.5	^a 0.074
Serum iron (μmol/L)	13.8±6.2	18.0±6.5	0.068
Serum iron saturation (%)	28.1±12.0	35.4±11.3	0.085
Serum ferritin (ug/L)	496.3±335.0	328.5±302.3	^a 0.074

Transferrin (g/L)	2.0±0.4	2.1±0.5	0.698
Hemoglobin (g/L)	127.8±19.6	128.5±19.4	0.886
MRI			
PDFF (%)	5.8±4.2	3.3±2.4	^a 0.001*
R2 (s ⁻¹)	50.4±24.8	38.3±8.8	^a 0.034*

Variables are presented as mean ± standard deviation. *indicates significant difference. Data were compared by either Student's t-test or non-parametric Mann-Whitney U-test (^a indicates Mann-Whitney U-test). ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; GGT: gamma glutamyl transpeptidase; ALP: alkaline phosphatase; MRI: magnetic resonance imaging; PDFF: proton density fat fraction.

We have also corrected these in statistical description. The revised texts are highlighted in red in corresponding section in the *Revised manuscript*.

Comment 3: Paragraph PDFF and R2: Authors give the crude results of R2 values, it would be wise to give values of liver iron concentration (LIC) extrapolated from R2 values; conversely in the figure 2 authors could add a graph on LIC.

Response: Thank you for your suggestions. According to your recommendation, we have calculated the values of LIC extrapolated from R2 values based on the known iron calibration equation^[1, 2]. In addition, we also added a graph on LIC in Figure 2. In the cholelithiasis group, LIC ranged from 0.48 to 10.01 mg.g⁻¹ dry tissue. In the healthy group, LIC ranged from 0.21 to 2.18 mg.g⁻¹ dry tissue. Mean LIC values were significantly higher in the cholelithiasis group (2.21±2.17 mg.g⁻¹ dry tissue *vs* 1.22±0.49 mg.g⁻¹ dry tissue, *P*=0.034) than in the healthy group.

Revised manuscript (PDFF, R2 and Liver iron concentration (LIC), page 9, line 16-21): The values of LIC extrapolated from R2 values were calculated based on the known iron calibration equation^[29,30]. In the cholelithiasis group, LIC ranged from 0.48 to 10.01 mg.g⁻¹ dry tissue. In the healthy group, LIC ranged from 0.21 to 2.18 mg.g⁻¹ dry tissue. Mean LIC values were significantly higher in the

cholelithiasis group ($2.21 \pm 2.17 \text{ mg.g}^{-1}$ dry tissue *vs* $1.22 \pm 0.49 \text{ mg.g}^{-1}$ dry tissue, $P=0.034$) than in the healthy group.

(Abstract: Results: page 3, line 25-27): Liver iron concentration (LIC) extrapolated from R2 values were significantly higher in the cholelithiasis group ($2.21 \pm 2.17 \text{ mg.g}^{-1}$ dry tissue *vs* $1.22 \pm 0.49 \text{ mg.g}^{-1}$ dry tissue, $P=0.034$) than in the healthy group.

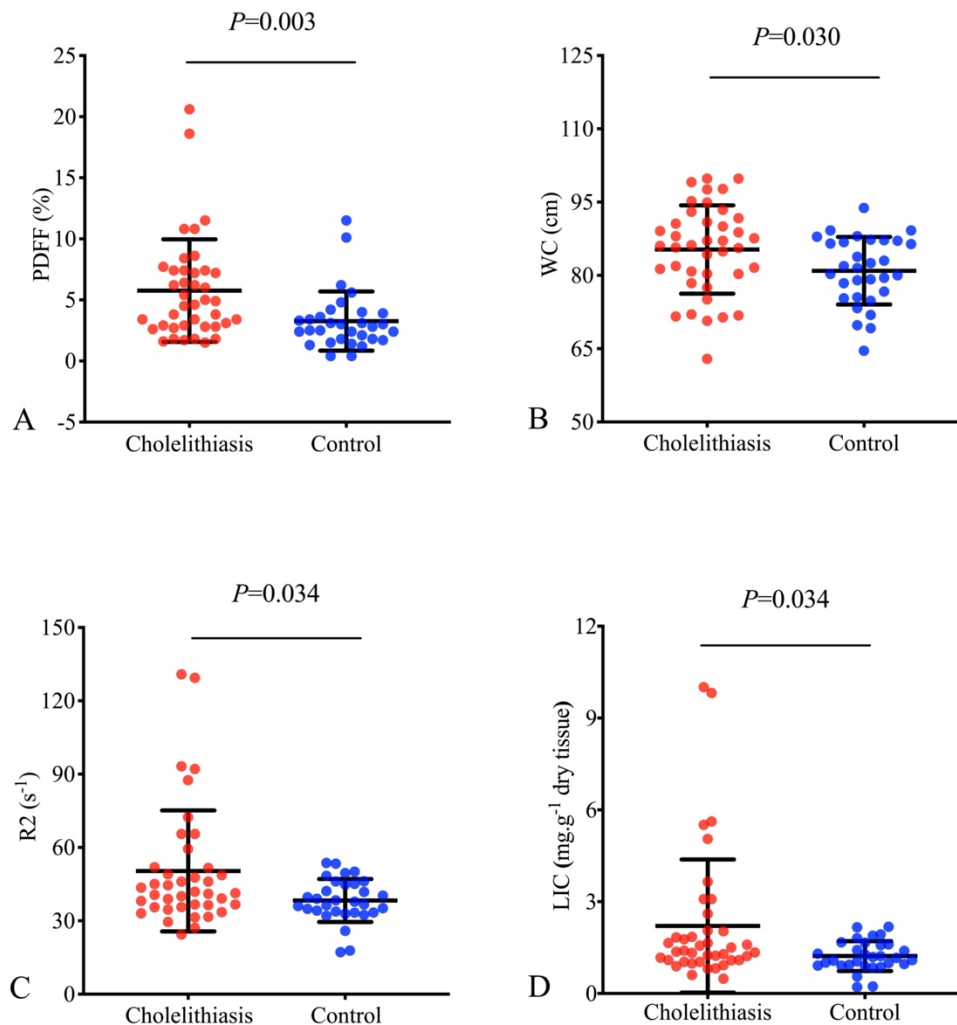


Figure 2 Graphs show PDFF (A), WC (B), R2 (C) and LIC (D) values in the cholelithiasis group and healthy controls. Variables are presented as mean \pm standard deviation. PDFF: proton density fat fraction; WC: waist circumference; LIC: liver iron concentration.

Comment 4: In the Discussion section: Authors should introduce this section on the mild magnitude of increase in PDFF and LIC in patients with cholelithiasis beside the diagnostic interest

of PDFF and possibly its pathophysiological role.

Response: Thank you for this valuable comment. Per suggestion, we have introduced in the Discussion section the mild magnitude of increase in PDFF and LIC in patients with cholelithiasis besides diagnostic interest of PDFF and possibly its pathophysiological role. In our study, patients with cholelithiasis had mild magnitude of increase in PDFF and LIC than normal controls. Previous study indicated that iron overload has the ability to synergistically upregulate the level of ferritin and fat accumulation in an intact organism, *C. elegans*, thus providing experimental evidence supporting the link between iron and obesity^[3].

Revised manuscript (Discussion, page 12, line 1-4): In our study, patients with cholelithiasis had mild magnitude of increase in PDFF and LIC than normal controls. Iron overload has the ability to synergistically upregulate the level of ferritin and fat accumulation in an intact organism, e.g. C. elegans, thus providing experimental evidence supporting the link between iron and obesity^[41].

Comment 5: Pages 10 and 11: authors discuss the data published on the relationship between PDFF and iron. Their discussion should include the recently published mechanistic demonstration of ability of iatrogenic iron overload in dialysis patients to induce an increase of liver fat fraction as its regression which parallels the normalization of LIC (ROSTOKER G, LORIDON C, GRIUNCELLI M, RABATE C, LEPEYTRE F, URENA-TORRES P, ISSAD B, GHALI N, COHEN Y. Liver Iron Load Influences Hepatic Fat Fraction in End-Stage Renal Disease Patients on Dialysis: A Proof of Concept Study. EBioMedicine. 2019 Jan;39:461-471. doi: 10.1016/j.ebiom.2018.11.020. Epub 2018 Nov 2).

Response: Thank you for your suggestion. Per suggestion, we have included the recently published study on the effects of iron in patients on dialysis^[4]. The published study investigated dialysis patients routinely receiving erythropoiesis-stimulating agents (ESA) and iron therapy by means of a non-invasive liver MRI^[4]. Rostoker et al^[4] demonstrated that liver iron load influences hepatic fat fraction in dialysis patients, besides, iron overload induced by iron therapy may aggravate or trigger nonalcoholic fatty liver disease (NAFLD) in dialysis patients. This study investigated the mechanism of the ability of iatrogenic iron overload in dialysis patients to induce an increase of

liver fat fraction as its regression which parallels the normalization of LIC. The results also show that iron products may have an adverse effect on the pathophysiology of NAFLD in non-renal patients, making the epidemic worse.

Revised manuscript (Discussion, page 12, line 4-12): In our study, patients with cholelithiasis had mild magnitude of increase in PDFF and LIC than normal controls. Iron overload has the ability to synergistically upregulate the level of ferritin and fat accumulation in an intact organism, e.g. *C. elegans*, thus providing experimental evidence supporting the link between iron and obesity^[41]. In addition, a previous study has also demonstrated that liver iron load influences hepatic fat fraction in dialysis patients who routinely received erythropoiesis-stimulating agents (ESA) and iron therapy, and iron overload induced by iron therapy may aggravate or trigger nonalcoholic fatty liver disease (NAFLD) in dialysis patients^[42]. This study confirmed the ability of iatrogenic iron overload in dialysis patients to induce an increase of liver fat fraction and its regression with the normalization of LIC. Taken together, iron products may have an adverse effect on the pathophysiology of NAFLD .

We tried our best to improve the manuscript and made changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list all the changes but marked in red in revised paper.

We appreciate for Editors and Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

REFERENCE

- 1 Lin H, Fu C, Kannengiesser S, Cheng S, Shen J, Dong H, Yan FH. Quantitative analysis of hepatic iron in patients suspected of coexisting iron overload and steatosis using multi-echo single-voxel magnetic resonance spectroscopy: Comparison with fat-saturated multi-echo gradient echo sequence. *Journal of Magnetic Resonance Imaging* 2018; **48**: 205-213 [PMID: 29513377 DOI: 10.1002/jmri.25967]
- 2 St Pierre TG, Clark PR., Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P,

Robins E, Lindeman. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005; **105**: 855-861 [PMID: 15256427 DOI: 10.1182/blood-2004-01-0177]

3 **Wang H**, Jiang X, Wu JY, Zhang LQ, Huang JF, Zhang YR, Zou XJ, Liang B. Iron Overload Coordinately Promotes Ferritin Expression and Fat Accumulation in *Caenorhabditis elegans*. *Genetics* 2016; **203**: 241-253 [PMID: 27017620 DOI: 10.1534/genetics.116.186742]

4 **Rostoker G**, Loridon C, Griuncelli M, Rabaté C, Lepeytre F, Ureña-Torres P, Issad B, Ghali N, Cohen Y. Liver Iron Load Influences Hepatic Fat Fraction in End-Stage Renal Disease Patients on Dialysis: A Proof of Concept Study. *EBioMedicine* 2019; **39**: 461-471 [PMID: 30502056 DOI: 10.1016/j.ebiom.2018.11.020]