Author’s response to reviews

Title: Novel equation to determine the hepatic triglyceride concentration in humans by MRI: diagnosis and monitoring of NAFLD in obese patients before and after bariatric surgery

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Version:2 Date:18 July 2014

Author's response to reviews: see over
Dear Editor,

Please find enclosed the revised version of our manuscript #3094298201279725 entitled “Novel equation to determine the hepatic triglyceride concentration in humans by MRI: diagnosis and monitoring of NAFLD in obese patients before and after bariatric surgery” by Jiménez-Agüero R, Emparanza JI, Beguiristain A, Bujanda L, Alustiza JM, García E, Hijona E, Gallego L, Sánchez J, Perugorria MJ, Asensio JI, Larburu S, Garmendia M, Larzabal M, Portillo MP, Aguirre L and Banales JM, which is resubmitted for its consideration for publication in *BMC Medicine*.

We greatly appreciate the Reviewers’ and Editor’s positive comments. Moreover, we believe we have adequately addressed each concern raised by the Reviewers and Editor, responding point-by-point to their comments and carrying out the required changes in the text (please find bellow the responses to Editor and Reviewers). For ease of review, all changes are presented underlined and in blue color in the revised version.

Thank you in advance for considering our manuscript.

Sincerely,

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RESPONSES TO EDITOR AND REVIEWERS:

We appreciate the thoughtful comments and suggestions given by the Reviewers and Editor, being aware that they were very helpful to improve the manuscript, we have tried to deal effectively with each of them.

Peer Reviewer Comments (Editor)

As you will see, the reviewers are generally impressed with your study, and suggest it is both timely, interesting and innovative. However both reviewers have suggested a number of revisions which will improve the potential impact of your study.

We acknowledge the Editor for her positive comments.

We would be grateful if you could address the comments in a revised manuscript clearly indicating any changes made, and provide a cover letter giving a point-by-point response to the concerns. Please note that further consideration of your manuscript is dependant on your responses to the reviewers comments. In order to be suitable for BMC Medicine, you must ensure that you fully adhere to the STARD guidelines (http://www.stard-statement.org/). Additionally, you must revise your discussion and abstract to reduce repetition and ensure that you focus on the most novel aspects of your work. Please note that if you do not change the focus of your discussion to include the most novel aspects, your manuscript would be better suited to a more specialist journal within the BMC Series, rather than BMC Medicine, since BMC Medicine research articles must be a significant clinical advance within the field.

As required, we have fully adhered our manuscript to the STARD guidelines and completed the “STARD checklist for reporting studies of diagnostic accuracy” (document attached). Moreover, we have modified both discussion and abstract in order to reduce repetitions and focus on the most novel aspects of our work. We believe that this new version of the manuscript suites properly to BMC Medicine, giving a significant clinical advance in the diagnostic and monitoring of NAFLD by magnetic resonance.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We have created our manuscript following the BMC Medicine journal style.
Peer reviewer #1: Prof. Joost P Drenth

Reviewer’s report:

The authors state that there is a quest for a novel non invasive procedure to estimate hepatic triglyceride content as the current gold standard liver biopsy with histopathological examination of cross-liver sections and the semi-quantitative estimation of the percentage of macrovesicular fat is cumbersome. To some extent I agree, and the authors have attempted to address this issue by evaluate the potential of multi-echo MRI in an unique set of patients, those subjected to bariatric surgery. Multi-echo MRI was performed in all patients and compared with a liver biopsy obtained at surgery. The novelty comes with the replication study where the evaluate their initial findings and ultimately the did a follow-up study in a sample from their cohort.

This is a fine paper that addresses an important issue and deserves wider dissemination. This article contributes to clinical advances in the diagnosis of NAFLD. Additionally, it contains an original equation to predict the hepatic triglyceride concentration. The methods of the study are elaborately described which makes a replication study possible. The conclusions based on the data are interesting and innovative. The article gives a detailed description of the performed study. However, it also raises some questions which I would like to present the authors.

- Many of this issues that are discussed below could be dealt with by completeing the http://www.stard-statement.org/ STARD Statement STAndards for the Reporting of Diagnostic accuracy studies. As such this study is a diagnostic accuracy study (a study that evaluated the ability of a test to differentiate between patients who have the target condition and those who do not have the target condition). I suggest that the authors use this algorithm and complete the checklist.

  We acknowledge this comment and have now adhered our manuscript to the STARD guidelines and completed the “STARD checklist for reporting studies of diagnostic accuracy” (document attached). On the other hand, the checklist’s items 13, 18, 19 and 21-24 are not applicable for our study. Moreover, the “flow diagram algorithm” is not valid for our study since MRI and Folch are continuous variables present in all individuals.

- The authors state in the methods section that “obese” patients are included that have a BMI > 40kg/m2. However, these patients have to be considered “morbid obese”. Table 1 shows a mean BMI of 44.6±7.9 kg. This indicates that also patients are included in the “obese group” with a BMI < 40kg/m2. Can the authors please elaborate on this?

  We appreciate this comment. The reviewer is right. “Morbid obese group” is now changed by “obese group” and includes patients with a BMI≥35. All this information is now clarified in the Methods section.

- When performing bariatric surgery the liver is manipulated to create a safe working space and view when stapling the stomach to perform the gastroenterostomy in a RYGB and resection of 2/3 of the stomach in a sleeve gastrectomy. This can possibly affect the local anatomy and make interpretation of the MRI post surgery more difficult. The method section describes that the MRI-biopsy period was less than 24h. Are all MRIs performed prior to surgery, or also post surgery? If MRIs are performed after surgery, does this affect the outcome parameter?

  All MRIs were performed the day before bariatric or liver surgery. We appreciate this comment and have clarified this important information in the Methods section.
- The final paragraph of the “Patient selection and study design” in the methods section states that 56 of the 97 obese patients and 11 patients who underwent a liver resection, were monitored one year after the operation. Are the authors able to give the characteristics of the patients who were not monitored after one year. Were they lost to follow-up or did a selection of patients take place?

From the 97 obese patients, all that had liver resection (n=11) and those who underwent bariatric surgery one year before (n=56) were monitored with a second multi-echo MRI one year after operation in order to quantitate steatosis. In this regard, since it is a prospective study, 30 patients did not fulfill the requirement of a year after bariatric surgery and had not undergone the second multi-echo MRI. All this valuable information is now stated in the Methods and Results sections.

- The multi-echo MRI is an interesting diagnostic method. However, this might be hard to interpret. This is an important issue as this determines how viable this method really is. How many people performed and interpret the results of the multi-echo MRI? Is every radiologist able to interpret these results or do they have a learning curve? Please elaborate on potential inter-observer differences in the MRI results.

Native multi-echo images are not directly analyzed by the radiologist. The software automatically generates the water and fat intensity maps from the native images. Water and fat maps are then analyzed as a conventional parametric map [region of interest (ROI) analysis] to calculate the final fat fraction measured as percentage. To avoid any bias, the final reported values were performed as a result of the average of three different ROIs. Therefore, the results are not subject to inter-observer bias. To clarify this important point, we have now included the aforementioned information in the Methods and Discussion sections.

- Due to the invasiveness a Folch measurement is not ethical 12 months after surgery. However, this would be the gold standard for monitorization of decreasing NAFLD after bariatric surgery. Additionally, this would also be the best method to check the accuracy of the developed equation. This should be discussed in the article.

We completely agree with the reviewer. When we considered a new biopsy one year after the surgery we found ethical problems for inherent risks. On the other hand, the results obtained in the "validation step" allowed us to extrapolate the results obtained in the "second RM" and calculate the “Folch value estimated” by the formula described in this manuscript.

As importantly indicated by the reviewer, all this information is now included in the Discussion section of the article.

- The conclusion of the authors states that multi-echo MRI may represent a low-cost non-invasive method to diagnose and monitor steatosis. Are the authors able to estimate the total costs of this method and compare it with Folch measurement?

We indicate below our costs of MRI and biochemical quantification of Folch:

- MR liver: 180 euros.
- Folch: ultrasound-guided liver biopsy (216 euros) + Folch determination (10 euros) = 226 euros.
Therefore, MRI, in addition to be a non-invasive technique, is cheaper than the Folch determination. This information is now included in the Discussion section of the manuscript.

- The authors introduce an interesting line of thinking: I cite “These data, ….Folch and semi-quantitative measurement of steatosis by histopathological examinations indicated that the latter (histopathological examinations) is not an accurate method to determinate the hepatic fat content, and Folch should be used as an appropriate gold standard for NAFLD”. Take a step back. The histopathology is the gold standard. This was used to validate the MRI and the FOLCH method and in the end the authors conclude that Folch is better than their gold standard. Am I missing something here? The gold standard is histology and everything that is compared will score less than 100%.

The measurement of steatosis by histopathological examinations has been considered the gold standard for the determination of NAFLD. In this regard, several recent reports showed different correlation values between “MRI fat fraction and histopathological examination” (Refs. [1, 10, 17, 22, 23] of the manuscript). However, the grade of steatosis by histopathology is estimated on a 0-3 scale and has inherent inter-observers bias, while the Folch is the direct quantification of the triglyceride concentration. Thus, we have shown here that the histopathological examination does not accurately correlate with the Folch. In addition, our correlation studies between “MRI fat fraction and Folch” were more significant than those of “MRI fat fraction and histopathological examinations”. Therefore, we may conclude that the Folch should be used as the appropriate gold standard for NAFLD, and based on our new formula, we can accurately determine its value by using multi-echo MRI.

All this important information is now fully explained in the Discussion section of the manuscript.

The text could be condensed to some extent. The article contains several paragraphs which repeat previous sections. The second paragraph of the discussion is a duplicate of the introduction. Additionally, the section “Our results from…estimated Folch values” in the discussion (page 12), is earlier stated in the first paragraph of the discussion. Finally, the section “Obese patients showed increased hepatic steatosis compared to controls by histopathological examination” contains the same information as the legend of figure 1. Please limit the repetitions.

We appreciate this comment and have now modified the manuscript accordingly.

Minor comments:

Monitorization is that a proper English term? Monitoring would be more appropriate.
The legend of Figure 5 states “MIR” in stead of “MRI”

Thanks. We have modified these words in the revised version of the manuscript.

Quality of written English: Needs some language corrections before being Published

The new version of the manuscript has been reviewed by a native American professional.

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
No competing conflict of interest

Peer reviewer #2: Prof. Jimmy Bell

In this study the author proposed a new equation for the estimation of liver Tg in NFLD. This is a timely paper as it tackles a key issue with the assessment of liver fat by MRI/MRS. However, much of the other data that the authors present in this manuscript has been previously published by other groups. Indeed there is a vast literature based on MRI (and MRS most of which the author appear to completely ignore) showing the value of MR techniques to assess NFLD in vivo.

Major Compulsory revision:

The focus of the paper should be completely changed given that the key result is the MRI vs Folch comparison which has not previously appeared in the literature. The other results simply confirm the potential clinical value of this.

We appreciate these comments. As suggested, we have changed the focus of the manuscript emphasizing our new observations on “MRI vs Folch”.

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests