PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (http://bmjopen.bmj.com/site/about/resources/checklist.pdf) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between Visceral Obesity and Hepatitis C Infection Stratified by Gender: A Cross-sectional Study in Taiwan
AUTHORS	Tsao, Yu-Chung; Chen, Jau-Yuan; Yeh, Wei-Chung; Peng, Yun- Shing; Li, Wen-Cheng

VERSION 1 - REVIEW

REVIEWER	Tyler A Bosch
	University of Minnesota USA
REVIEW RETURNED	25-Apr-2017

GENERAL COMMENTS	The objective of this study is not well defined and should be clarified in the introduction, the statement is made that "we would like to find out if there are differences by gender", this should be constructed into a more refined question.
	The methods section is lacking, the authors relied on cut-points from CT and MRI however, the agreement between BIA and those methods is not great which could result in over or under representation of visceral obesity.
	The authors performed several t-tests but there is no mention of adjusting for multiple comparisons.
	The three body composition variables chosen by the authors are all roughly the same, and definitely related, some justification of why these ratios were chosen is necessary.
	Given the small sample of HCV positive when multivariate logistic regression models may have been overfit, more information to this regard is necessary for interpretation of the results.
	It is unclear how the results of this study tie in with several portions of the discussion section. Please revise this accordingly based on the attached file.

REVIEWER	Giada Sebastiani McGill University
REVIEW RETURNED	27-Apr-2017

GENERAL COMMENTS	This retrospective and cross-sectional study by Li and colleagues
	investigates the gender difference of visceral obesity among HCV

infected patients as compared to HCV neg cases (total number of
cases=1267). The authors found that classical factors (body fat percent, fat free mass/BW and muscle mass/BW) were independent determinants for visceral obesity in HCV neg patients, but not in HCV pos patients. The conclusions of the authors is that HCV modulates host lipid metabolism and distribution.
Overall, this is a descriptive work and the retrospective and nature fo the study puts it at risk for biases. It is difficult to provide a mechanistic statement as the one present at the end of the abstract and in the discussion. Moreover, critical virological variables which can influence lipid metabolism are lacking, including HCV RNA, antiviral treatment status and HCV genotype. The authors provide speculation in both introduction and discussion about the role of HCV in modulating host lipid metabolism and the role of different HCV genotypes and/or HCV replication in this process, but they fail to provide these critical information. Finally, the effect of sex is not adjusted for in multivariable analysis, but rather a stratification analysis is done, and an invariable analysis regarding differences among females and males among HCV pos patients are not provided. As such, it is unclear to me why the title is focused on the role of sex and the research question mentions that this study is focusing on the gender difference of visceral obesity among HCV pos patients.
 Specific comments Strength and limitations: second last sentence of this section is not appropriate. As said above, authors do not provide a mechanistic explanation (so they are not actually clarifying how HCXV modulates host lipid metabolism) but only an association between known metabolic factors and visceral obesity, broke down by gender and HCV status. Introduction: the authors state epidemiology of HCV in the world population, but some information for the specific geographic setting of Taiwan should also be provided. Results: HCV pos patients are a small sample size as compared to HCV neg. Please note some typos (page 9 "circumstance" is not right). Discussion: the first statements are not focusing on the results of this study, but on modulation of HCV in lipid metabolism. The second sentence does not contain the object (independent determinant of what?)

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 Reviewer Name: Tyler A Bosch Institution and Country: University of Minnesota USA Please state any competing interests: None declared

Please leave your comments for the authors below

Comment#1: The objective of this study is not well defined and should be clarified in the introduction, the statement is made that "we would like to find out if there are differences by gender", this should be constructed into a more refined question.

Comment#2: The methods section is lacking, the authors relied on cut-points from CT and MRI however, the agreement between BIA and those methods is not great which could result in over or under representation of visceral obesity.

Response#2: Thank you for the comment. We highly agree that in assessing visceral fat area, computed tomography (CT) is still adopted as the gold standard. We had reviewed articles with a recent study cross-validating the regression equation and obtained a strong correlation between BIA and CT for estimating visceral fat area (Ref 13). We had added this in our limitation part in the discussion. (Page 16, Paragraph 3) However, BIA still got its strength, which is non-invasive, relatively inexpensive, does not expose to ionizing radiation, has very limited between observer variations and can be performed in almost any subject because it is portable. Nevertheless, we look forward to designing further study to confirm the trend in our study by CT-estimating visceral fat area.

Comment#3: The authors performed several t-tests but there is no mention of adjusting for multiple comparisons.

Response#3: Thank you very much for this precious comment. According to your recommendation, we combined Table 2 and 3 into Table 2. Because there were multiple comparisons in terms of several t-tests, we therefore lower the alpha level using Bonferroni adjustment method. For example, the alpha level would be set as 0.0028 (0.05 / 18) if there were 18 tests. (Page 9, Paragraph 3; Page 10, Paragraph 3) Besides, to study the risk factors of visceral obesity in the total patients, we conducted a multivariable logistic regression analysis with a backward elimination method. (Table 3) (Page 9, Paragraph 3; Page 11, Paragraph 2)

Comment#4: The three body composition variables chosen by the authors are all roughly the same, and definitely related, some justification of why these ratios were chosen is necessary. Response#4: As we observed in Table 5, body fat percentage was positively related with visceral obesity while fat free mass/BW and muscle mass/BW was in the contrary. As you mentioned, muscle mass is related to fat free mass because it is one of the parts that make up overall fat free mass. Fat free mass refers to the total body weight minus all the weight of fat mass. Muscle mass is indeed a different type of mass from fat free mass. These three body composition variables had different clinical implements.

Comment#5: Given the small sample of HCV positive when multivariate logistic regression models may have been overfit, more information to this regard is necessary for interpretation of the results. Response#5: Thank you for your suggestion. Since we observed that Body fat percentage, Fat free mass/BW and Muscle mass/BW were significant associating factors in the Table 4.

We built several multivariable regression models to identify the difference between patients with and without HCV infection.

Comment#6: It is unclear how the results of this study tie in with several portions of the discussion section. Please revise this accordingly based on the attached file.

Response#6: Thank you for pointing out this, we had revised our discussion and had been making sure that all the sections tie in the results.

Reviewer: 2 Reviewer Name: Giada Sebastiani Institution and Country: McGill University Please state any competing interests: None declared

Please leave your comments for the authors below

This retrospective and cross-sectional study by Li and colleagues investigates the gender difference of visceral obesity among HCV infected patients as compared to HCV neg cases (total number of cases=1267).

The authors found that classical factors (body fat percent, fat free mass/BW and muscle mass/BW) were independent determinants for visceral obesity in HCV neg patients, but not in HCV pos patients. The conclusions of the authors is that HCV modulates host lipid metabolism and distribution.

Comment#7: Overall, this is a descriptive work and the retrospective and nature fo the study puts it at risk for biases. It is difficult to provide a mechanistic statement as the one present at the end of the abstract and in the discussion.

Response#7: As you mentioned, due to retrospective setting of the current study, it is indeed not appropriate for us to jump into the conclusion. A well-designed prospective study is necessary to support the trend we found in this study. Therefore, we modified the title, abstract and discussion accordingly with a view to fit our statement with the study design better.

Comment#8: Moreover, critical virological variables which can influence lipid metabolism are lacking, including HCV RNA, antiviral treatment status and HCV genotype. The authors provide speculation in both introduction and discussion about the role of HCV in modulating host lipid metabolism and the role of different HCV genotypes and/or HCV replication in this process, but they fail to provide these critical information.

Response#8: Thank you for pointing out this. Because this was a cross-sectional study of retrospective nature, we did not have data regarding HCV RNA, or follow-up data for HCV RNA. Persistent anti-HCV positive status or assaying HCV RNA is typically used to confirm chronic hepatitis; however, it was not possible to do that in this retrospective, cross-sectional study. We would like to add this in our limitation and look forward to design future study including detailed virological variables you mentioned. Among major genotypes, HCV genotype 1 is prevalent around the world. In Taiwan, HCV subtypes 1b and 2a are the major subtypes (Ref 39). (Page 17, Paragraph 1)

Comment#9: Finally, the effect of sex is not adjusted for in multivariable analysis, but rather a stratification analysis is done, and an invariable analysis regarding differences among females and males among HCV pos patients are not provided. As such, it is unclear to me why the title is focused on the role of sex and the research question mentions that this study is focusing on the gender difference of visceral obesity among HCV pos patients.

Response#9: Thank you for your suggestion. Since we observed that Body fat percentage, Fat free mass/BW and Muscle mass/BW were significant associating factors in both genders (Table 4). Therefore, we built several multivariable regression models to identify the difference between patients with and without HCV infection in different genders (Table 5).

We found the trend was not so obvious in patients with HCV infection. Furthermore, body fat percentage was not even a significant determinant for HCV infected females when adjusting for confounding factors.

Comment#10: Specific comments

1) Strength and limitations: second last sentence of this section is not appropriate. As said above, authors do not provide a mechanistic explanation (so they are not actually clarifying how HCXV modulates host lipid metabolism) but only an association between known metabolic factors and visceral obesity, broke down by gender and HCV status.

2) Introduction: the authors state epidemiology of HCV in the world population, but some information for the specific geographic setting of Taiwan should also be provided.

3) Results: HCV pos patients are a small sample size as compared to HCV neg. Please note some typos (page 9 "circumstance" is not right).

4) Discussion: the first statements are not focusing on the results of this study, but on modulation of

HCV in lipid metabolism. The second sentence does not contain the object (independent determinant of what?)

Response#10:

1) We modified the "Strength and limitations" section accordingly.

2) We provided the information in the introduction as you recommended. (Page 7, Paragraph 1)

3) Thank you very much for pointing out this. We corrected them accordingly.

4) Thank you for the comment. We modified the statement and corrected the grammar.

(Supplementary file 1)

VERSION 2 – REVIEW

REVIEWER	Tyler A Bosch
	University of Minnesota
	United States
REVIEW RETURNED	16-Jun-2017

GENERAL COMMENTS	The authors did and excellent job of addressing the majority of the concerns and comments I raised. However, the primary result and discussion point within this study is about association of body fat percentage/ fat-free mass/ BW and muscle mass / body weight with risk of visceral adiposity. The authors make the statement that the relation of these variables within HCV positive is not as clear and there may also be a gender component involved.
	Yet the evidence that is used to support this claim is questionable because the statistical methods used are flawed. The multivariate models built are very likely over fit because of the small number of total sample size in the HCV positive group and an even smaller number of visceral obesity individuals within that group. In females only 13 of 45 individuals were classified with visceral adiposity and in the males group only 7 of 59 were not classified with visceral adiposity. The general rule would be 15 observations within each group (ex. 15 with visceral adiposity 15 without) for every independent variable within the model. Yet in model 3 there are at least 6 independent variables included within the model. Thus, it seems that the conclusion may be presumptuous given that the weight of evidence would suggest that HCV positive does not influence visceral adiposity in males or females. The subgroups had similar means for all areas in males and females, %BF FFM/BW and Muscle mass/BW were all significantly associated in univariate analyses. This is further complicated by using a methodology for estimated visceral fat area.
	The authors should provide some evidence to the fit of the model and that adding in the additional covariates improved the model fit dramatically.
	The data is interesting, but I have concerns that the primary conclusion relies on 1 statistical model that is flawed and the overall weight of the evidence suggests the opposite.

REVIEWER	Giada Sebastiani
	McGill University
	Canada
REVIEW RETURNED	13-Jun-2017

GENERAL COMMENTS The authors have answered the main concerns relative to this paper. The title is more effective, discussion improved, quality of English now good.	e to this paper. of English
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 2 Reviewer Name: Giada Sebastiani Institution and Country: McGill University, Canada Please state any competing interests: None declared

Comment#1: The authors have answered the main concerns relative to this paper.

The title is more effective, discussion improved, quality of English now good. Response#1: Thank you for your previous suggestions.

Reviewer: 1 Reviewer Name: Tyler A Bosch Institution and Country: University of Minnesota, United States Please state any competing interests: None

The authors did and excellent job of addressing the majority of the concerns and comments I raised. However, the primary result and discussion point within this study is about association of body fat percentage/ fat-free mass/ BW and muscle mass / body weight with risk of visceral adiposity.

The authors make the statement that the relation of these variables within HCV positive is not as clear and there may also be a gender component involved.

Comment#2: Yet the evidence that is used to support this claim is questionable because the statistical methods used are flawed. The multivariate models built are very likely over fit because of the small number of total sample size in the HCV positive group and an even smaller number of visceral obesity individuals within that group. In females only 13 of 45 individuals were classified with visceral adiposity and in the males group only 7 of 59 were not classified with visceral adiposity. The general rule would be 15 observations within each group (ex. 15 with visceral adiposity 15 without) for every independent variable within the model. Yet in model 3 there are at least 6 independent variables included within the model. Thus, it seems that the conclusion may be presumptuous given that the weight of evidence would suggest that HCV positive does not influence visceral adiposity in males or females. The subgroups had similar means for all areas in males and females, %BF FFM/BW and Muscle mass/BW were all significantly associated in univariate analyses. This is further complicated by using a methodology for estimated visceral fat area.

The authors should provide some evidence to the fit of the model and that adding in the additional covariates improved the model fit dramatically.

The data is interesting, but I have concerns that the primary conclusion relies on 1 statistical model that is flawed and the overall weight of the evidence suggests the opposite.

Response#2: Thanks for your valuable comments. After careful consideration, we really acknowledged that our multivariable logistic model was not appropriate, especially in the HCV-infective group due to the very small sample size. We strongly agree with your opinion that our

original logistic model (Model 3) had obvious problem of "overfitting", especially in the HCV-infective group. Therefore, we performed a series likelihood-ratio test to determine what variable(s) can significantly improve the model fit in HCV-infective males and females. Finally, we found only age provides some contribution (statistically significant) to the fit of the model, therefore we decided to solely adjust for age in the revised Model 2 (Page 25, Table 5). Therefore, we revised the statistical methods (Page 9, Paragraph 1), results (Page 11, paragraph 1), and discussion (Page 11, Paragraph 2; Page 15, Paragraph 2, 3) as well as abstract (Page 4, Paragraph 1) accordingly.

VERSION 3 – REVIEW

REVIEWER	Tyler A Bosch University of Minnesota, USA
REVIEW RETURNED	21-Jul-2017

GENERAL COMMENTS	The authors did a good job of addressing the statistical concerns