PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The dose-response association between physical activity and non-alcoholic fatty liver disease: a case-control study in a Chinese population
AUTHORS	li, yangfan; He, Fei; he, yun; pan, xinting; wu, yunli; hu, zhijian; lin, xu; xu, shanghua; Peng, Xian-E

VERSION 1 - REVIEW

REVIEWER	Goh Eun Chung Seoul National University Hospital, Korea
REVIEW RETURNED	15-Oct-2018

GENERAL COMMENTS	The manuscript entitled "The dose-response association between physical activity and non-alcoholic fatty liver disease: a case-control study in a Chinese population." investigated the association between physical activity and NAFLD in a Chinese population, and drew a conclusion that physical activity was inversely associated with the risk of NAFLD in a dose-dependent manner. The manuscript was written clearly. However, there are some major concerns that the authors should address. 1) A dose-dependent trend between physical activity and NAFLD is previously well-known as you cited, [Liver Int. 2015 Mar;35(3):944-52]. Thus, the novelty of this manuscript is limited. 2) How about insulin resistance or HOMA-IR? 3) FFQ involves many variables regarding nutrition. However, authors analyzed only total energy intake. How about other variables such as carbohydrate, total fat or protein? 4) How about the prevalence of hypertension or diabetes? 5) The diagnosis of fatty liver using ultrasonography has limitations. Authors should discuss about the limitation. 6) Because authors used frequency matching according to age and gender, age and gender should be deleted in the co-variables in the multivariate analyze model.

REVIEWER	Kannan Sridharan Arabian Gulf University
REVIEW RETURNED	21-Nov-2018

GENERAL COMMENTS	The diagnosis of NAFLD has to be made by the gold standard biopsy findings and not by USG. Also, the physical activity was assessed using questionnaire that is likely to be associated with recall bias and inaccuracies. There is no mention on what basis

	was the sample size chosen? Hence, the results of this study may not be accurate for the said group of patients. Further, the authors have not carefully considered adherence to STROBE guidelines. There is mismatch in several STROBE items with regard to what they have mentioned in the checklist compared to the manuscript.

REVIEWER	Sadeq A Al-Maweri
	Al-Farabi Colleges
REVIEW RETURNED	04-Dec-2018

GENERAL COMMENTS	Dear authors,
	This piece of work is quite interesting, well- conducted and nicely written. However there are several points that should be addressed:
	- In page 2 line 7, the word "development" is little confusing and so I would suggest to be replaced by another expression such as "prevention"
	- Methods section:
	- No information was given regarding sample size calculation.
	- Variables including outcomes, exposures and potential confounders should be clearly stated, preferably under separate subheadings.
	- Language errors:
	There are numerous typos and grammatical errors throughout the text. The manuscript may benefit from English editing.
	Thank you

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Thank you very much for the careful reading of our manuscript and the valuable suggestions. We have carefully considered the comments and have revised the manuscript accordingly. Detailed response as follows:

Q1：A dose-dependent trend between physical activity and NAFLD is previously well-known as you cited, [Liver Int. 2015 Mar;35(3):944-52]. Thus, the novelty of this manuscript is limited.

A1：Many thanks for your comment. Indeed, a dose-dependent trend between physical activity and NAFLD have been reported, however, the effects of physical activity on NAFLD in Chinese population is unclear. Furthermore, physical activity is a complex concept including the type, intensity, frequency and duration. In many studies, only frequency of physical activity was considered which could not concisely reflect the dose of physical activity. In addition, the data of physical activity was retrieved from populations with diverse demographic characteristics. Therefore the optimal intensity and dose of physical activity for the treatment of NAFLD have yet to be elucidated. American Association for the Study of Liver Diseases suggested that Moderate-intensity exercise is good for hepatic steatosis, but the optimal duration and intensity of exercise remain undetermined[The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2017 Jul 17 [Epub].https://doi.org/10.1002/hep.29367.]. European Association for the Study of the Liver-European Association for the Study of Diabetes-European Association for the Study of Obesity recommended that Moderate-intensity aerobic physical activity in

3–5 sessions for a total of 150–200 min/week is generally preferred, and resistance training is also effective. Physical activity has a dose-response relationship, and vigorous rather than moderate exercise carries the full benefit for NASH and fibrosis [EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016; 64:1388-1402.]. Korean Association for the Study of the Liver advised that Exercise more than twice per week and for more than 30 minutes is beneficial for reducing hepatic steatosis [KASL clinical practice guidelines: management of non alcoholic fatty liver disease. Clin Mol Hepatol 2013; 19:325-348.]. The study population was heterogeneous, meaning that the results should be interpreted with caution and that optimal dose of physical activity should be tailored to the patient's clinical characteristics, fitness status and preferences. Thus we explore the dose-response association between physical activity and NAFLD in a Chinese population.

In addition, several potential confounding variables, including energy intake and sedentary time, were taken into account in the present study. With the development of technology and a better economy, people tend to spend more time in sedentary activities: one study showed that sitting time was positively associated with risk of NAFLD, even in subjects with a high level of physical activity. Similarly, another study indicated that regular participation in high levels of physical activity does not fully protect against the risks associated with prolonged bouts of sedentary behaviors. Other known risk factors of NAFLD are energy intake and BMI. Several previous studies have found that NAFLD patients tend to have higher energy intake, and a restricted-energy diet was found to have great benefits for weight loss and improving BMI. The potential confounding effect of these factors may reduce the power to detect associations between physical activity and the risk of NAFLD. Thus we considered sedentary time and energy intake at the same time when investigating the association between physical activity and NAFLD.

Q2: How about insulin resistance or HOMA-IR?

A2: Many thanks for your comment. Many studies have revealed that insulin resistance or HOMA-IR plays a key role in NAFLD. In our study, all subjects were recruited from a health examination center underwent routine health checks. In our country, biochemical examination is one program of routine physical examination, including fasting blood glucose testing, but does not include insulin testing. Usually only people who are suspected or diagnosed with diabetes will be tested for serum insulin levels.   Therefore, most people only tested fasting blood glucose, but not insulin levels. Thus, we collected information on the history of diabetes through a structured questionnaire. The prevalence of diabetes in the NAFLD group was 4.8%, and the control group was 2.2%. In NAFLD group, serum levels of fasting blood glucose were also higher than in the control population (p< 0.001) (please see table1).Thus in further analysis, we adjusted for the diabetes and fasting blood glucose in multivariate logistic model.

Q3: FFQ involves many variables regarding nutrition. However, authors analyzed only total energy intake. How about other variables such as carbohydrate, total fat or protein?

A3: We agree with the reviewer's concerns and constructive suggestion. We recalculated the distribution of the three energy nutrients (carbohydrate, fat and protein) in cases and controls in the revised manuscript (please see tableS3 and tableS4). In the section of result, we adjusted for the carbohydrate, total fat and protein in the multivariate logistic model (please see tableS5, tableS6). After adjusting for these variables, the association between physical activity and NAFLD was maintained in males. However daily diets contain a variety of foods, not individual nutrients or individual foods, and there are complex interactions between different nutrients or foods. Based on individual food or nutrient studies, the association between diet and NAFLD cannot be accurately assessed. Thus we finally analyzed only total energy intake in the finally multivariate logistic model.

Q4: How about the prevalence of hypertension or diabetes?

A4: Many thanks for your comment. The prevalence of hypertension in cases and controls were 30% and 18.8%, and the difference between two groups was statistically significant (p< 0.001). The prevalence of diabetes in cases and controls were 4.8% and 2.2%, and the difference between two groups was statistically significant (p=0.02) (please see table1). Many studies revealed that hypertension and diabetes were risk factors for NAFLD, thus we adjusted these variables in multivariate logistic model.

Q5: The diagnosis of fatty liver using ultrasonography has limitations. Authors should discuss about the limitation.

A5: We agree with the reviewer's concerns and constructive suggestion. We have discussed the limitations of diagnosis of fatty liver using ultrasonography in the section of discussion of the revised manuscript(please see page18).

Q6: Because authors used frequency matching according to age and gender, age and gender should be deleted in the co-variables in the multivariate analyze model.

A6: We agree with the reviewer's concerns and constructive suggestion. In the results section of the revised manuscript, age and gender have been deleted in the co-variables in the multivariate analyze model and we have revised the related description (please see table2 and table3).

Reviewer: 2

Thank you very much for your valuable comments. We have carefully thought about your comments and have made corresponding changes to the manuscript and a new revised submission have been uploaded.

Q1: The diagnosis of NAFLD has to be made by the gold standard biopsy findings and not by USG.

A1: Many thanks for your comment. Indeed, liver biopsy is the gold standard for quantitative diagnosis of NAFLD. However it is an invasive examination, there exist the possibility of postoperative blood and bile leakage, and there are sampling errors, therefore does not apply to routine screening. Ultrasound examination currently is the preferred method for the initial screening of NAFLD with its advantages of no scratching, no radiation damage, reproducibility and low price. It is based on the enhancement or attenuation of intrahepatic echo and the progression of intravascular blood vessels. In moderate to severe steatosis, the sensitivity and specificity of ultrasound diagnosis are high (78.4%~90.8% and 76.9%~90.9%, respectively). However, ultrasound diagnosis is susceptible to individual differences, checking instrument performance and parameter selection, operating experience and many other factors, so ultrasound quantitative diagnosis of fatty liver still has limitations. This diagnosis mainly depends on the subjective judgment of the operator, and there is no objective and unified quantitative index. And it is difficult to identify liver fibrosis and liver fat. Each method has its own advantages and disadvantages and we have discussed the limitations of diagnosis of fatty liver using ultrasonography in the section of discussion of the revised manuscript(please see page18). It is hoped that with the advancement of science and technology, better non-invasive diagnostic methods will emerge.

Q2: the physical activity was assessed using questionnaire that is likely to be associated with recall bias

A2: We agree with the reviewer's concerns and constructive suggestion. Selective participation and recall bias are potential concerns in our study as with other epidemiologic studies of this nature. Therefore, our study can only provide etiological clues in exploring the association between physical activity and NAFLD, and randomized controlled trial studies are therefore required for more accurate

results. We have discussed the limitations in the section. of discussion of the revised manuscript(please see page17).

Q3: There is no mention on what basis was the sample size chosen?

A3: We agree with the reviewer's concerns and suggestion.We have added the description about the calculation of sample size in the method section of the revised manuscript(please see page5).

Reviewer: 3

We are very appreciate you for careful reading of our manuscript and the valuable suggestions. We seriously thought about your comments and made changes to the manuscript.

Q1: In page 2 line 7, the word "development" is little confusing and so I would suggest to be replaced by another expression such as "prevention"

A1: We agree with the reviewer's concerns and suggestion. We have replaced the word "development" in page 2 line 7 by "prevention".

Q2: No information was given regarding sample size calculation.

A2: We agree with the reviewer's concerns and suggestion. We have added the description about the calculation of sample size in the method section of the revised manuscript (please see page5).

Q3: Variables including outcomes, exposures and potential confounders should be clearly stated, preferably under separate subheadings.

A3: We agree with the reviewer's concerns and suggestion. According to your suggestion, variables including outcomes, exposures and potential confounders were clearly stated under separate subheadings in the method section of the revised manuscript(please see pages from 5 to 8)

VERSION 2 – REVIEW

REVIEWER	Goh Eun Chung Korea, republic of
REVIEW RETURNED	16-Jan-2019

GENERAL COMMENTS	1. Please cite supplementary tables in the text . There is no citation of Table S4-S6. 2. Please discuss the different results according to the gender.

REVIEWER	Kannan Sridharan Arabian Gulf University
REVIEW RETURNED	12-Jan-2019

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

Reviewer: 1

Q1：Please cite supplementary tables in the text. There is no citation of Table S4-S6.

A1：Many thanks for your suggestion. We added a citation to the supplementary material on page 11, and analyzed and explained the table S4-S6.

Q2：Please discuss the different results according to the gender.

A2：Many thanks for your suggestion. In the section of discussion（page 15）, we discuss the different results according to the gender.