

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	I appreciate author responses. I have no additional recAssociation between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study in Chinaommentations/comments.
AUTHORS	Chen, Yang; Li, Jie; Li, Tianyu; Long, Jianxiong; Liao, Jinling; Wei, Gong-Hong; Mo, Zengnan; Cheng, Jiwen

VERSION 1 – REVIEW

REVIEWER	Onder Canguven Hamad General Hospital, Doha, Qatar
REVIEW RETURNED	29-Apr-2018

GENERAL COMMENTS	<p>Authors examined the association between homocysteine , vitamin B12, folic acid and erectile dysfunction. In your manuscript you mentioned “However, the definite pathogenesis is unclear.” for ED. There are many pathogenesis which was proven in the literature.</p> <p>You got results “From September to December 2009, including4303 men” Why did it take so long time to publish?</p> <p>You say “On the other hand, the function of B12 on ED is one interesting discussion.” This sentence is weird for a scientific paper, could you remove it please?</p> <p>In conclusion, You wrote “Meanwhile, B12 was also significantly associated with ED.” But earlier you also wrote “Although with three adjusted models, no significant results are detected for B12 in linear regression analysis.” Which is correct?</p>
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REVIEWER	Tomás Ahern Our Lady of Lourdes Hospital, Ireland
REVIEW RETURNED	14-May-2018

GENERAL COMMENTS	<p>Strengths</p> <ol style="list-style-type: none">1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total.3. Appropriate regression analyses were performed to assess for associations.
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	<p>Major problems</p> <ol style="list-style-type: none"> 1. The title does not make sense. What are “risk effects”? Using the term “effect” in the description of a cross-sectional study is not appropriate. The term “effect” suggests causality, which cannot be determined in a cross-sectional study. 2. The standard of written English is poor. A lot of sentences did not make sense – it was difficult to try to understand what the points of many sentences were. 3. Use of the term “samples” is confusing. Does this term refer to blood samples or to participants? The term “sample” would suggest the former. But it appears that the authors used it to describe the latter. 4. No “subgroup” analyses were performed. Regression and between-group analyses were performed. 5. The term “unsatisfactory marital status” is unclear and possibly judgemental. 6. Conclusions were included in the results section of the abstract. 7. Incorrect statements were made in the results section of the abstract. These included “vitamin B12 is identified to increase ED risk”. This statement does not make sense. Did the authors mean to state that a low vitamin B12 level is associated with an increased prevalence of ED? If so, no data to support this statement have been provided. Did the authors mean to state that vitamin B12 levels were lower in men with ED? If so, the relationship did not persist after multivariate adjustment. Another incorrect statement was “Moreover, along with the increase of B12, the risk effect enhanced.” This statement does not make sense. I do not see any data to support any possible interpretation of this statement. 8. Incorrect statements were made in the conclusions section of the abstract. These included “In summary, HCY might be the risk factor of ED.” It is impossible to determine whether a parameter is a risk factor without performing a longitudinal analysis. Another incorrect statement was “And B12 is significantly associated with ED development”. It is impossible to comment on the development of a condition without performing a longitudinal study. 9. The authors stated, on multiple occasions, that relationships existed despite the statistical significance of the association being no longer present after adjustment for potential confounders. This is not appropriate. 10. The authors stated, in the first paragraph of the discussion among other places, that “HCY is detected to increase the risk of ED.” This statement suggests that the authors feel that homocysteine causes ED. Causality cannot be assessed with a cross-sectional study. <p>Minor problems</p> <ol style="list-style-type: none"> 1. Abstract. The study’s design was not indicated in the abstract. 2. Introduction. No pre-specified hypothesis was stated. 3. Methods and Materials. It is unclear how long after initial examination that participants responded to further interviews. It is unclear whether blood was taken on the same day as the initial routine physical examination or on the same day as a later interview or on a separate day altogether. 4. Methods and Materials. A table is required to compare those who participated with those who chose not to. 5. Methods and Materials. It is unclear that informed consent to study participation was provided by the study subjects. Absolute clarity is required for this important issue.
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	<p>6. Methods and Materials. It appears that a “comprehensive questionnaire” was involved in this study which was “mainly performed by the trained investigators using a standardised protocol.” What proportion of the subjects had a comprehensive questionnaire performed? What proportion of the subjects had a comprehensive questionnaire performed by a non-trained investigator or by a non-investigator?</p> <p>7. Methods and Materials. Why was ED classified into five groups instead of the conventional six?</p> <p>8. Methods and Materials. All diseases which were used to make up the exclusion criteria need to be stated. This list can be included in the supplemental section.</p> <p>9. Methods and Materials. All drugs which were used to make up the exclusion criteria need to be stated. This list can be included in the supplemental section.</p> <p>10. Methods and Materials. The numbers of participants who were screened, who were examined, who had blood taken and who made up the analytical sample needs to be stated clearly in the results section. Ideally this would be with the use of a flowchart.</p> <p>11. Methods and Materials. How were variables tested for Gaussian distribution?</p> <p>12. Methods and Materials. The authors need to explain why they included both BMI and WHR in the list of co-variates. BMI and WHR are both measures of abdominal obesity.</p> <p>13. Methods and Materials. The authors need to explain why they chose to perform quartile analysis and not quintile analysis.</p> <p>14. Methods and Materials. Did the authors correct for multiple testing?</p> <p>15. Methods and Materials. What did the authors use as their threshold for significance?</p> <p>16. Methods and Materials. What was the power of the study to detect the main association of interest?</p> <p>17. Methods and Materials. How were missing data handled?</p> <p>18. Results. The vitamin B12 level was associated with mild ED, but not with more severe forms of ED. Do the authors have a possible, biologically plausible, explanation for this?</p> <p>19. Discussion. The majority of the second paragraph of the discussion should not be in the discussion section. It would be better placed in the introduction section – the text sets the context for the study.</p> <p>20. Table 1. It is unclear how the p-values were obtained. Was a different test performed for “FA” to “Marital status”. How was marital status defined?</p> <p>21. Table 2. Data regarding regression co-efficient values should be in one table. Data regarding odds ratio values should be in a separate table. Having regression co-efficients and odds ratios in the same table is confusing and makes it difficult to compare parameter association strengths.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Onder Canguven

Institution and Country: Hamad General Hospital, Doha, Qatar

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

Authors examined the association between homocysteine, vitamin B12, folic acid and erectile dysfunction.

1. In your manuscript you mentioned "However, the definite pathogenesis is unclear." for ED. There are many pathogenesis which was proven in the literature.

Answer: We are very sorry for our improper description. In our revised paper, we have removed this sentence.

2. You got results "From September to December 2009, including 4303 men" Why did it take so long time to publish?

Answer: Thank you for your significant question. Our Fangchenggang Area Male Health and Examination Survey (FAMHES) project was launched in 2009, which was mainly designed to understand the relationship between environmental and genetic factors. In this project, we had got the data about the International Index of Erectile Function (IIEF-5) score, NIH-chronic prostatitis symptom index (NIH-CPSI), etc., and the biochemical indexes (such as blood routine, hepatorenal function, homocysteine, vitamin B12, folic acid, hormone, etc.). The details were shown previously [1]. In fact, we did not pay attention to the association between the homocysteine (HCY), vitamin B12, folic acid (FA) and erectile dysfunction (ED) at the beginning. Recently, some clues suggested that there might be some potential effects of HCY, B12 and FA in ED [2-4]. So, this time, in order to understand their associations, we performed this study on the basis of our FAMHES project.

1. Chen Y, Li J, Hu Y, Zhang H, Yang X, Jiang Y, Yao Z, Chen Y, Gao Y, Tan A, Liao M, Lu Z, Wu C, Xian X, Wei S, Zhang Z, Chen W, Wei GH, Wang Q, Mo Z. Multi-factors including Inflammatory/Immune, Hormones, Tumor-related Proteins and Nutrition associated with Chronic Prostatitis NIH IIIa+b and IV based on FAMHES project. *Sci Rep.* 2017;7(1):9143.
2. Khan MA, Thompson CS, Emsley AM, et al. The interaction of homocysteine and copper markedly inhibits the relaxation of rabbit corpus cavernosum: new risk factors for angiopathic erectile dysfunction? *BJU Int.* 1999;84:720-4.
3. Zhang Z, Xu Z, Dai Y, et al. Elevated serum homocysteine level as an independent risk factor for erectile dysfunction: a prospective pilot case-control study. *Andrologia* 2016. doi: 10.1111/and.12684.
4. Sansone M, Sansone A, Romano M, et al. Folate: a possible role in erectile dysfunction? *Aging Male.* 2017:1-5.

3. You say "On the other hand, the function of B12 on ED is one interesting discussion." This sentence is weird for a scientific paper, could you remove it please?

Answer: Thank you for your suggestion. We have removed this sentence.

4. In conclusion, You wrote "Meanwhile, B12 was also significantly associated with ED." But earlier you also wrote "Although with three adjusted models, no significant results are detected for B12 in linear regression analysis." Which is correct?

Answer: We are sorry to confuse you. In our study, baseline, linear and logistic regression analyses were applied to evaluate the association between the homocysteine (HCY), vitamin B12, folic acid (FA) and erectile dysfunction (ED). In our baseline analysis, HCY was identified to be significantly associated with ED. Then, the linear regression analysis was conducted, in which the IIEF-5 scores were treated as the dependent factor. After adjusting the covariates, no significant results were

detected for B12. Even though, we furtherly performed the binary logistic regression analysis, in which the status of ED (ED: IIEF-5 \leq 21; Non-ED: IIEF-5 $>$ 21) was treated as the dependent factor. Then, B12 was identified to be associated with ED in the Unadjusted model (OR=1.438, 95%CI=1.070-1.933, P=0.016). (Table 3) Furtherly, we tried to discover the relationship between B12 and ED, based on the severity grades of ED. Interestingly, B12 was confirmed to be related to ED especially among mild ED (Unadjusted: OR=1.694, 95%CI=1.207-2.376, P=0.002; Age-adjusted: OR=1.596, 95%CI=1.135-2.244, P=0.007; Multivariate adjusted: OR=1.620, 95%CI=1.141-2.300, P=0.007). (Table 4) Then, the level of B12 was divided into quartiles. The result suggested that along with the increases of B12 level, the association between B12 and ED enhanced (Unadjusted: Q2: OR=0.917, P=0.569; Q3: OR=0.988, P=0.939; Q4: OR=1.452, P=0.015; P for trend $<$ 0.001). (Table 5) Our analyses suggested that age could not be ignored in investigating the relationship in the B12 and ED. So, we grouped the participants into four parts (ages $<$ 40, 40-49, 50-59 and \geq 60 years). Our results showed that the significant association between B12 and mild ED (IIEF-5=17–21) mainly presents in 40-49 years (OR=2.907, 95%CI=1.402-6.026, P=0.004). (Table S2)

In summary, except for linear regression, our binary and multinomial logistic regression analyses all discovered the significant association between B12 level and ED. So, the significant relationship in B12 and ED cannot be ignored.

In order to make the descriptions clearer, we have revised the sentences in the “Results” sections and table legends.

Reviewer: 2

Reviewer Name: Tomás Ahern

Institution and Country: Our Lady of Lourdes Hospital, Ireland

Please state any competing interests or state ‘None declared’: None

Please leave your comments for the authors below

Strengths

1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.
2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total.
3. Appropriate regression analyses were performed to assess for associations.

Answer: Thank you for your reviews.

Major problems

1. The title does not make sense. What are “risk effects”? Using the term “effect” in the description of a cross-sectional study is not appropriate. The term “effect” suggests causality, which cannot be determined in a cross-sectional study.

Answer: Thank you for your significant advice. We have revised our title as “Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study”. Additionally, we also checked all whole the paper to replace the term “effect” with appropriate words.

2. The standard of written English is poor. A lot of sentences did not make sense – it was difficult to try to understand what the points of many sentences were.

Answer: Thank you for your advice. We are sorry for our poor English. In order to make the new revised article more fluent and understandable, we had sent it to the professional institute with native

English speakers for polishing the grammars. We hope that the new manuscript would satisfy you and the audiences.

3. Use of the term “samples” is confusing. Does this term refer to blood samples or to participants? The term “sample” would suggest the former. But it appears that the authors used it to describe the latter.

Answer: Thank you for your significant correction. We have revised all the ambiguous “samples” in our paper.

4. No “subgroup” analyses were performed. Regression and between-group analyses were performed.

Answer: Thank you for your significant correction. We have revised the descriptions in the new paper.

5. The term “unsatisfactory marital status” is unclear and possibly judgemental.

Answer: We are sorry for our unclear descriptions. This time, we have specified the “unsatisfactory marital status” as “the men who lives alone (spinsterhood or widowed)” in our revised paper.

6. Conclusions were included in the results section of the abstract.

Answer: We are sorry for the repetitive descriptions in the “Conclusions” and “Results” section of the abstract. We have amended our abstract to avoid the improper sentence in the two different sections.

7. Incorrect statements were made in the results section of the abstract. These included “vitamin B12 is identified to increase ED risk”. This statement does not make sense. Did the authors mean to state that a low vitamin B12 level is associated with an increased prevalence of ED? If so, no data to support this statement have been provided. Did the authors mean to state that vitamin B12 levels were lower in men with ED? If so, the relationship did not persist after multivariate adjustment. Another incorrect statement was “Moreover, along with the increase of B12, the risk effect enhanced.” This statement does not make sense. I do not see any data to support any possible interpretation of this statement.

Answer: We are very sorry to confuse you with our unclear descriptions. In our baseline analysis, vitamin B12 level was higher in the ED group (718.53 ± 234.37) compared to the Non-ED group (688.74 ± 229.68 , $P = 0.015$). In order to understand the association between B12 and ED, the logistic regression analysis was conducted, in which binary ED was the dependent factor. Then, results confirmed that B12 was significantly associated with ED (OR = 1.438, 95% CI = 1.070-1.933, $P = 0.016$), although this correlation disappeared after adjusting for multiple factors. Furthermore, we divided the level of B12 into quartiles to discuss the association between B12 and ED along with the increase of B12. Our results also suggested that B12 might be significantly related to ED, especially at the higher levels (Unadjusted: Q2: OR = 0.917, $P = 0.569$; Q3: OR = 0.988, $P = 0.939$; Q4: OR = 1.452, $P = 0.015$; P for trend < 0.001).

As you have stated, causality cannot be assessed with a cross-sectional study. So, these two sentences were improper. This time, we have revised them to make it more suitable.

8. Incorrect statements were made in the conclusions section of the abstract. These included “In summary, HCY might be the risk factor of ED.” It is impossible to determine whether a parameter is a risk factor without performing a longitudinal analysis. Another incorrect statement was “And B12 is significantly associated with ED development”. It is impossible to comment on the development of a condition without performing a longitudinal study.

Answer: Thank you for your significant advices. We are very sorry for our improper terms in describing our results and conclusions. We have checked all whole the paper to modify these words and sentences.

9. The authors stated, on multiple occasions, that relationships existed despite the statistical significance of the association being no longer present after adjustment for potential confounders. This is not appropriate.

Answer: Thank you for your significant suggestion. In order to investigate the association between HCY, B12, FA, and ED comprehensively, baseline analysis, linear and logistic regression analyses, and multinomial logistic regression analysis were included. In the baseline analysis, HCY and B12 levels were identified to be significantly different in the ED and Non-ED groups ($P < 0.05$). Then, regression and between-groups analyses were performed for the association between HCY, B12, and ED. For HCY, fragmentary proofs suggested that it might be related to ED. As for B12, although in the binary logistic regression analysis B12 is identified to be associated with ED, this correlation disappeared after adjusting for potential confounders, especially age. So, we grouped the participants into four age groups (ages < 40 , $40-49$, $50-59$, and ≥ 60 years old). Our results showed that the significant correlation between B12 and mild ED (IIEF-5 = 17-21) mainly presents in the 40-49 year old age group (OR = 2.907, 95% CI = 1.402-6.026, $P = 0.004$) after multivariate adjustment. In addition, the positive correlation between B12 and ED was also confirmed, especially among men with mild ED even after adjustment for potential confounders. These results may suggest that the positive association between B12 and ED was true in some extent.

10. The authors stated, in the first paragraph of the discussion among other places, that “HCY is detected to increase the risk of ED.” This statement suggests that the authors feel that homocysteine causes ED. Causality cannot be assessed with a cross-sectional study.

Answer: We are sorry for our improper illustrations. In our study, we also detected that HCY might be associated with ED as previous studies. We have checked the whole paper to avoid the incorrect sentences.

Minor problems

1. Abstract. The study's design was not indicated in the abstract.

Answer: We are sorry for the missing information in the abstract. Our study was designed as the cross-sectional study. This time, we have added it in the abstract definitely.

2. Introduction. No pre-specified hypothesis was stated.

Answer: We are sorry for the missing pre-specified hypothesis. This time, we have added the descriptions as “On the basis of previous studies, we hypothesize that there might be a true association between HCY, B12, FA, and ED.”

3. Methods and Materials. It is unclear how long after initial examination that participants responded to further interviews. It is unclear whether blood was taken on the same day as the initial routine physical examination or on the same day as a later interview or on a separate day altogether.

Answer: We are sorry to confuse you with our unclear descriptions. In fact, the initial examination, further interviews, and blood samples were obtained on the same day. First, we explained the aim, possible results, and requirements (such as initial examination, further interviews, and blood samples) of our project to the participants coming for routine physical examination at the Medical Center in

Fangchenggang First People's Hospital one by one. It should be noted that these participants were asked to fast for at least 8 h (overnight) the day before.

In the morning, between 8:00 am and 11:00 am, they were invited to participate in this project. Once the participants made full sense of our study and were willing to take part, they were asked to sign the written informed consents. At this time, blood samples were collected from the voluntary participants. These blood samples were transported to the Department of Clinical Laboratory at the First Affiliated Hospital of Guangxi Medical University in Nanning within 2-3 h, where they were centrifuged within 15-25 min and stored at -80 °C.

Whether or not willing to provide the blood samples, all the participants signing the written informed consents were asked to complete the initial examination (including height, weight, waistline, and hipline) and further interviews including essential information, such as age, sex, smoking, and drinking, and disease-related scores (NIH-Chronic Prostatitis Symptom Index, International Prostate Symptom Score, and International Index of Erectile Function, etc.).

The flow of this process is shown in Figure S1.

4. Methods and Materials. A table is required to compare those who participated with those who chose not to.

Answer: Thank you for your significant suggestion. From September 2009 to December 2009, 4303 men coming for routine physical examination at the Medical Center in Fangchenggang First People's Hospital were enrolled. Among them, 3593 participants responded for further interviews (response rate = 83.5%) [1]. Further interviews gathered essential information (e.g., age, sex, smoking, and drinking) and disease-related scores (NIH-Chronic Prostatitis Symptom Index, International Prostate Symptom Score, and International Index of Erectile Function). Although we tried our best to invite all 4303 men to take part in these interviews, about 710 of them still refused to provide complete information, which made it hard to compare their essential features. However, according to the biochemical indexes in routine physical examinations at the hospital, currently, these 710 men are in health statuses similar to the 3593 participants without obvious abnormality.

1. Chen Y, Xin X, Zhang H, et al. Immunization associated with erectile dysfunction based on cross-sectional and genetic analyses. PLoS One 2014; 9:e111269.

5. Methods and Materials. It is unclear that informed consent to study participation was provided by the study subjects. Absolute clarity is required for this important issue.

Answer: We are sorry to confuse you. In fact, we have stated this important issue as "Written informed consents were signed by all participants" in the "Population and data collection" section.

6. Methods and Materials. It appears that a "comprehensive questionnaire" was involved in this study which was "mainly performed by the trained investigators using a standardised protocol." What proportion of the subjects had a comprehensive questionnaire performed? What proportion of the subjects had a comprehensive questionnaire performed by a non-trained investigator or by a non-investigator?

Answer: Thank you for your question. We are sorry to confuse you with our unclear descriptions. After acquiring the permission and written informed consents from the participants, all of them were invited to accomplish a comprehensive questionnaire with a face-to-face interview performed by the trained investigators using a standardized protocol. In order to make it clearer, we have revised the sentence in the "Population and data collection" section.

7. Methods and Materials. Why was ED classified into five groups instead of the conventional six?

Answer: Thank you for your question. We are very sorry that we did not understand your question well. In our study, according to the IIEF-5 score, the symptoms of ED were mainly divided into four groups: None (IIEF-5= 22-25), Mild (17-21), Moderate (12-16) and Severe (5-11) [1].

1. Kupelian V, Araujo AB, Chiu GR, Rosen RC, McKinlay JB. Relative contributions of modifiable risk factors to erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. *Prev Med.* 2010 Jan-Feb;50(1-2):19-25.

8. Methods and Materials. All diseases which were used to make up the exclusion criteria need to be stated. This list can be included in the supplemental section.

Answer: Thank you for your significant advice. We have added the excluded diseases in the Table S1.

9. Methods and Materials. All drugs which were used to make up the exclusion criteria need to be stated. This list can be included in the supplemental section.

Answer: Thank you for your significant advice. We have added the excluded drugs in the Table S1. All the drugs which might affect the HCY, B12 and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine, or glucocorticoids, were excluded. In other words, once the participants were currently taking drugs belonging to these categories, they would be removed.

10. Methods and Materials. The numbers of participants who were screened, who were examined, who had blood taken and who made up the analytical sample needs to be stated clearly in the results section. Ideally this would be with the use of a flowchart.

Answer: Thank you for your significant advice. In order to make the flow for screening the eligible participants clearer, we have added a flowchart in the Figure S2.

11. Methods and Materials. How were variables tested for Gaussian distribution?

Answer: We are sorry for the missing descriptions for the methods to test for Gaussian distribution. Before analysis, HCY, B12, FA levels were tested for Gaussian distribution with the Shapiro-Wilks test. Then, they were logarithmically transformed, in order to ensure the approximate Gaussian distribution. The descriptions were shown in the "Statistical analysis" section.

12. Methods and Materials. The authors need to explain why they included both BMI and WHR in the list of co-variates. BMI and WHR are both measures of abdominal obesity.

Answer: Thank you for your question. BMI and WHR are the indexes applied to estimate obesity. Among them, BMI tends to evaluate body fatness but has a weak ability to differentiate fatness as central or visceral [1]. Alternatively, WHR is said to be more effective in reflecting the visceral fat and central adiposity but is not suitable for an estimation of body fat [1, 2]. So, to some extent, combining BMI and WHR will help to understand obesity more comprehensively. Additionally, the predictive effects of BMI and WHR in diseases are different [3, 4]. Above all, although both BMI and WHR are used to evaluate obesity, their functions and emphasis are different. So, in our study, these two indexes are used as the covariates.

1. McDonnold M, Mele LM, Myatt L, Hauth JC, Leveno KJ, Reddy UM, Mercer BM; Eunice Kennedy Shriver National Institute of Child Health Human Development Maternal-Fetal Medicine Units (MFMU)

Network. Waist-to-Hip Ratio versus Body Mass Index as Predictor of Obesity-Related Pregnancy Outcomes. *Am J Perinatol.* 2016 May;33(6):618-24.

2. Suchanek P, Kralova Lesna I, Mengerova O, Mrazkova J, Lanska V, Stavek P. Which index best correlates with body fat mass: BAI, BMI, waist or WHR? *Neuro Endocrinol Lett.* 2012;33 Suppl 2:78-82.

3. Tang B, Han CT, Zhang GM, Zhang CZ, Yang WY, Shen Y, Vidal AC, Freedland SJ, Zhu Y, Ye DW. Waist-hip Ratio (WHR), a Better Predictor for Prostate Cancer than Body Mass Index (BMI): Results from a Chinese Hospital-based Biopsy Cohort. *Sci Rep.* 2017 Mar 8;7:43551.

4. Welborn TA, Dhaliwal SS. Preferred clinical measures of central obesity for predicting mortality. *Eur J Clin Nutr.* 2007 Dec;61(12):1373-9.

13. Methods and Materials. The authors need to explain why they chose to perform quartile analysis and not quintile analysis.

Answer: Thank you for your significant question. In our opinion, it is both rational to perform either quartile analysis or quintile analysis with enough participants. Generally, quartile analysis for HCY, B12 and FA is applied in other studies [1-2]. So, in our study, we also choose to perform the quartile analysis.

1. Ng TP, Feng L, Niti M, Kua EH, Yap KB. Folate, vitamin B12, homocysteine, and depressive symptoms in a population sample of older Chinese adults. *J Am Geriatr Soc.* 2009 May;57(5):871-6.

2. Tamai Y, Wada K, Tsuji M, Nakamura K, Sahashi Y, Watanabe K, Yamamoto K, Ando K, Nagata C. Dietary intake of vitamin B12 and folic acid is associated with lower blood pressure in Japanese preschool children. *Am J Hypertens.* 2011 Nov;24(11):1215-21.

14. Methods and Materials. Did the authors correct for multiple testing?

Answer: Thank you for your question. In fact, we did not correct for multiple testing at first. This time, Bernoulli correction was applied, with the significant threshold of $P < 0.0125$ ($= 0.05/4$ tests) for multinomial logistic regression analysis. However, as you can see, no positive P values were shown, which suggested that the association between B12 and ED was unstable. And further studies with larger numbers of participants are needed urgently. In order to describe this more clearly, we have added the illustrations in our "Discussion", "Limitations", and "Conclusions" sections.

15. Methods and Materials. What did the authors use as their threshold for significance?

Answer: We are sorry for the descriptions of the threshold for significance. The threshold for significance is $P < 0.05$. In the revised paper, we have added the sentence in "Statistical analysis" section.

16. Methods and Materials. What was the power of the study to detect the main association of interest?

Answer: Thank you for your significant question. In our study, we confirmed the significant association between HCY and ED. In addition, interestingly, the positive correlation between B12 and ED was also identified, especially among patients with mild ED (Unadjusted: OR = 1.694, 95% CI = 1.207-2.376, $P = 0.002$; Age-adjusted: OR = 1.596, 95% CI = 1.135-2.244, $P = 0.007$; Multivariate adjusted: OR = 1.620, 95% CI = 1.141-2.300, $P = 0.007$). (Table 4) The means \pm standard deviations of B12 levels were 6.47 ± 0.38 (693 participants), 6.54 ± 0.33 (465 participants), 6.50 ± 0.36 (137 participants), and 6.46 ± 0.40 (86 participants) for the Non-ED (IIEF-5 score 22-25), mild ED (17-21), moderate ED (12-16), and severe ED (5-11) groups, respectively. On the basis of PASS software (NCSS, LLC), the statistical powers were calculated in which $\alpha = 0.05$; $\beta_0 = -3.828$ and $\beta_1 = 0.527$ in

the Unadjusted model; $\beta_0 = -4.562$ and $\beta_1 = 0.467$ in the Age-adjusted model; and $\beta_0 = -4.836$ and $\beta_1 = 0.482$ in the Multivariate adjusted model. Then, our results suggested the power could reach near 90%. Thank you very much for your significant guidance and advice again.

17. Methods and Materials. How were missing data handled?

Answer: Thank you for your question. In our study, the missing data was deleted in the analyses.

18. Results. The vitamin B12 level was associated with mild ED, but not with more severe forms of ED. Do the authors have a possible, biologically plausible, explanation for this?

Answer: Thank you for significant question. Although our study identifies the potential association between B12 and ED, ED tends to have high levels of B12 (ED: 718.53 ± 234.37 , Non-ED: 688.74 ± 229.68 , $P = 0.015$). Meanwhile, the significant association between B12 and ED was more dominant for mild ED at the higher B12 levels. There are two explanations. First, our results suggest that the function of B12 in ED might be dose-dependent. Excess B12 levels would increase the risk of mild ED with some unclear mechanisms. Second, increased B12 might provide negative feedback for this disease. At the beginning of the disease, a defense mechanism is triggered. As a potential protective factor, the absorption of B12 is enhanced. Combined with limited studies, our study can also propose that B12 is significantly associated with ED. As for the exact effects of B12 on ED, further studies are needed, which might pave the way for the treatment of ED with B12 in the future. The descriptions are shown in the “Discussion” section.

19. Discussion. The majority of the second paragraph of the discussion should not be in the discussion section. It would be better placed in the introduction section – the text sets the context for the study.

Answer: Thank you for your significant advice. This time, in the revised paper, we have moved the appropriate sentences in the “Introduction” section.

20. Table 1. It is unclear how the p-values were obtained. Was a different test performed for “FA” to “Marital status”. How was marital status defined?

Answer: We are very sorry to confuse you. We have explained the statistical tests and the definition of marital status in the Table 1.

21. Table 2. Data regarding regression co-efficient values should be in one table. Data regarding odds ratio values should be in a separate table. Having regression co-efficients and odds ratios in the same table is confusing and makes it difficult to compare parameter association strengths.

Answer: Thank you for your significant advice. We have separated Table 2 into two independent tables (Table 2-3).

VERSION 2 – REVIEW

REVIEWER	Tomás Ahern RCSI Hospital Group, Ireland
REVIEW RETURNED	01-Aug-2018
GENERAL COMMENTS	Strengths

	<p>1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.</p> <p>2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total.</p> <p>Major problems</p> <p>1. The standard of written English is poor. A lot of sentences did not make sense – it was difficult to try to understand what the points of many sentences were. I needed to read through the paper a number of times to understand the main points that were being made. The results and conclusions sections of the abstract need special attention.</p> <p>2. The term “spinsterhood” does not apply to men. Men who do not marry are “bachelors” or have a life of “bachelorhood”.</p> <p>Minor problems</p> <p>1. Methods and Materials. It is unclear how long after initial examination that participants responded to further interviews. It is unclear whether blood was taken on the same day as the initial routine physical examination or on the same day as a later interview or on a separate day altogether.</p> <p>2. Methods and Materials. It is unclear that informed consent to study participation was provided by the study subjects. Absolute clarity is required for this important issue. The current version of the manuscript states “written informed consents were signed by all participants.” The authors may wish to change this statement to “All participants signed a form indicating that they had provided their informed consent to study participation.”</p> <p>3. Methods and Materials. It should be stated that data that were not Gaussian in distribution were logarithmically transformed to achieve Gaussian distribution. I assume that not all data were transformed. If data were already Gaussian in distribution, there would be no need to logarithmically transform them. This should be clarified.</p> <p>4. Methods and Materials. The authors need to explain why they included both BMI and WHR in the list of co-variables. BMI and WHR are both measures of abdominal obesity.</p> <p>5. Methods and Materials. Did the authors correct for multiple testing? A statement answering this question needs to be placed in the Methods and Materials section.</p> <p>6. Methods and Materials. How were missing data handled? A statement answering this question needs to be placed in the Methods and Materials section.</p> <p>7. Table 4. The legend suggests that multinomial logistic regression analyses were used. I assume that this multiple logistic regression analyses? If so, what were the categorical dependent variables? If it was the various ED groups, based on the IIEF-5, this needs to be stated in the table legend.</p> <p>8. Table 5. What was the categorical dependent variable? If it was ED treated as a binary categorical variable, this needs to be stated in the table legend.</p>
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VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 2

Reviewer Name: Tomás Ahern

Institution and Country: RCSI Hospital Group

Ireland

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

Strengths

1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.
2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total.

Answer: We thank you for your encouraging comments on our study.

Major problems

1. The standard of written English is poor. A lot of sentences did not make sense – it was difficult to try to understand what the points of many sentences were. I needed to read through the paper a number of times to understand the main points that were being made. The results and conclusions sections of the abstract need special attention.

Answer: We agree and have made proper correction and rewording as described above, point #2 by the Editor. In addition, we have revised our “Abstract” section according to the reviewer’s comments #9.

2. The term “spinsterhood” does not apply to men. Men who do not marry are “bachelors” or have a life of “bachelorhood”.

Answer: Thank you for your corrections. We have checked the whole paper and revised these descriptions accordingly.

Minor problems

1. Methods and Materials. It is unclear how long after initial examination that participants responded to further interviews. It is unclear whether blood was taken on the same day as the initial routine physical examination or on the same day as a later interview or on a separate day altogether.

Answer: We are sorry to confuse you with our unclear descriptions again.

In fact, the initial examination, further interviews, and blood samples were obtained on the same day. First, we explained the aim, possible results, and requirements (such as initial examination, further interviews, and blood samples) of our project to the participants coming for routine physical examination at the Medical Center in Fangchenggang First People's Hospital one by one. It should be noted that these participants were asked to fast for at least 8 h (overnight) the day before.

In the morning, between 8:00 am and 11:00 am, they were invited to participate in this project. Once the participants made full sense of our study and were willing to take part, they were asked to sign the written informed consents. At this time, blood samples were collected from the voluntary participants. These blood samples were transported to the Department of Clinical Laboratory at the First Affiliated Hospital of Guangxi Medical University in Nanning within 2-3 h, where they were centrifuged within 15-25 min and stored at -80 °C.

Whether or not willing to provide the blood samples, all the participants signing the written informed consents were asked to complete the initial examination (including height, weight, waistline, and hipline) and further interviews including essential information, such as age, sex, smoking, and drinking, and disease-related scores (NIH-Chronic Prostatitis Symptom Index, International Prostate Symptom Score, and International Index of Erectile Function, etc.).

The flow of this process is shown in Figure S1.

This time, in order to make it clear in the paper, we have added the clear description in the "Population and data collection" section, as "These processes above including initial examination (including height, weight, waistline, and hipline), further interviews (essential information, such as age, sex, smoking and drinking, etc.), and blood collection, were performed on the same days coherently."

2. Methods and Materials. It is unclear that informed consent to study participation was provided by the study subjects. Absolute clarity is required for this important issue. The current version of the manuscript states "written informed consents were signed by all participants." The authors may wish to change this statement to "All participants signed a form indicating that they had provided their informed consent to study participation."

Answer: Thank you for your significant advice. We have revised this sentence as "All participants signed a form indicating that they had provided their informed consent to study participation." in the "Population and data collection" section.

3. Methods and Materials. It should be stated that data that were not Gaussian in distribution were logarithmically transformed to achieve Gaussian distribution. I assume that not all data were transformed. If data were already Gaussian in distribution, there would be no need to logarithmically transform them. This should be clarified.

Answer: Thank you for your suggestion. In the revised paper, we have modified the description as “If data were not Gaussian in distribution, they were logarithmically transformed, in order to ensure the approximate Gaussian distribution.”.

4. Methods and Materials. The authors need to explain why they included both BMI and WHR in the list of co-variates. BMI and WHR are both measures of abdominal obesity.

Answer: Thank you for your question again. BMI and WHR are the indexes applied to estimate obesity. Among them, BMI tends to evaluate body fatness but has a weak ability to differentiate fatness as central or visceral [1]. Alternatively, WHR is said to be more effective in reflecting the visceral fat and central adiposity but is not suitable for an estimation of body fat [1, 2]. So, to some extent, combining BMI and WHR will help to understand obesity more comprehensively. Additionally, the predictive effects of BMI and WHR in diseases are different [3, 4]. Above all, although both BMI and WHR are used to evaluate obesity, their functions and emphasis are different. So, in our study, these two indexes are used as the covariates. In order to explain this issue, we added the descriptions in the “Statistical analysis” section.

1. McDonnold M, Mele LM, Myatt L, Hauth JC, Leveno KJ, Reddy UM, Mercer BM; Eunice Kennedy Shriver National Institute of Child Health Human Development Maternal-Fetal Medicine Units (MFMU) Network. Waist-to-Hip Ratio versus Body Mass Index as Predictor of Obesity-Related Pregnancy Outcomes. *Am J Perinatol.* 2016 May;33(6):618-24.

2. Suchanek P, Kralova Lesna I, Mengerova O, Mrazkova J, Lanska V, Stavek P. Which index best correlates with body fat mass: BAI, BMI, waist or WHR? *Neuro Endocrinol Lett.* 2012;33 Suppl 2:78-82.

3. Tang B, Han CT, Zhang GM, Zhang CZ, Yang WY, Shen Y, Vidal AC, Freedland SJ, Zhu Y, Ye DW. Waist-hip Ratio (WHR), a Better Predictor for Prostate Cancer than Body Mass Index (BMI): Results from a Chinese Hospital-based Biopsy Cohort. *Sci Rep.* 2017 Mar 8;7:43551.

4. Welborn TA, Dhaliwal SS. Preferred clinical measures of central obesity for predicting mortality. *Eur J Clin Nutr.* 2007 Dec;61(12):1373-9.

5. Methods and Materials. Did the authors correct for multiple testing? A statement answering this question needs to be placed in the Methods and Materials section.

Answer: Thank you for your question. The Bernoulli correction was applied, with the significant threshold of $P < 0.0125$ ($= 0.05/4$ tests) for multinomial logistic regression analysis. The descriptions were added in the “Statistical analysis” section.

6. Methods and Materials. How were missing data handled? A statement answering this question needs to be placed in the Methods and Materials section.

Answer: Thank you for your question. In our study, the missing data was deleted in the analyses. This time, the statement was added in the “Statistical analysis” section.

7. Table 4. The legend suggests that multinomial logistic regression analyses were used. I assume that this multiple logistic regression analyses? If so, what were the categorical dependent variables? If it was the various ED groups, based on the IIEF-5, this needs to be stated in the table legend.

Answer: We are sorry to confuse you. Firstly, it is true that the “multinomial logistic regression analyses” was indicated as “multiple logistic regression analyses”. The categorical dependent variables were the various ED groups, based on the IIEF-5. In order to make it clear, it was stated in the table legend.

8. Table 5. What was the categorical dependent variable? If it was ED treated as a binary categorical variable, this needs to be stated in the table legend.

Answer: We are sorry to confuse you. In this analysis, The levels of HCY, B12, and FA was divided into quartile (Q175%), which were treated as the categorical dependent variables. And the Q1 was the reference. As a binary categorical variable, the ED was put as the “Factors”.

9. I suggest that the results and discussion section of the abstract be reworded as follows.

Results

No association between FA and ED was found. Significant correlations between HCY and ED were found – the relationships between these two parameters were most notable in men aged over 60 years and in men living alone. B12 levels were higher in men with ED (718.53±234.37 pg/ml vs 688.74±229.68, p=0.015). Using multinomial logistic regression analyses, B12 levels were related to mild ED (Multivariate adjusted analysis: OR = 1.620, 95% CI = 1.141-2.300, p=0.007), especially among men aged 40–49 years (OR = 2.907, 95% CI = 1.402-6.026, p=0.004).

Conclusions

We report, for the first time, a relationship between B12 levels and ED. We found also specific cohorts of men for whom the relationship between HCY levels and ED is most prominent. Further studies are required to elucidate the mechanisms underlying these relationships – these may ultimately result in new therapies for ED.

Answer: Thank you very much for your significant work for our paper. We have revised our “Abstract” section according to your fluent and refined languages. In addition, we also try our best to modify the sentences in the full text.

VERSION 3 – REVIEW

REVIEWER	Tomas Ahern Our Lady of Lourdes Hospital, Royal College of Surgeons of Ireland Hospital Group, Ireland.
REVIEW RETURNED	29-Oct-2018

GENERAL COMMENTS	<p>Strengths</p> <ol style="list-style-type: none">1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total. <p>Major problems</p> <ol style="list-style-type: none">1. None. <p>Minor problems</p> <ol style="list-style-type: none">1. Abstract. Should the words “were ascertained” be included after “HCY, B12 and FA” in the participants subsection?2. Conclusion. The sentence “Our results confirmed the pathogenic effect of HCY on ED, especially on severe ED” should be removed. An effect cannot be determined in an observational study. The term “pathogenic effect” suggests causality. Causality cannot be determined in an observational study.
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VERSION 3 – AUTHOR RESPONSE

Editors Comments to Author:

Reviewer: 2

Reviewer Name: Tomas Ahern

Institution and Country: Our Lady of Lourdes Hospital, Royal College of Surgeons of Ireland Hospital Group, Ireland.

Please state any competing interests or state ‘None declared’: None.

Please leave your comments for the authors below

Strengths

1. This study addressed an important issue – the possibility that readily micronutrient levels are associated with erectile dysfunction. Positive findings would provide a rationale toward interventional studies to assess whether micronutrient therapy could be used to treat this common complaint.
2. The study involved participants in the large Fangchenggang Area Male Health and Examination Survey (FAMHES) which recruited 4,303 men in total.

Major problems

1. None.

Minor problems

1. Abstract. Should the words “were ascertained” be included after “HCY, B12 and FA” in the participants subsection?

Answer: Thank you for your significant question. We have revised the “Participants” and “Measures” section as follows:

“Participants: A total of 1381 participants completed questionnaires were included, between September 2009 and December 2009.

Measures: ED was evaluated by the International Index of Erectile Function (IIEF-5) scores. And the values of HCY, B12 and FA were acquired. Then, Regression and between-group analyses were performed.”

2. Conclusion. The sentence “Our results confirmed the pathogenic effect of HCY on ED, especially on severe ED” should be removed. An effect cannot be determined in an observational study. The term “pathogenic effect” suggests causality. Causality cannot be determined in an observational study.

Answer: Thank you for your significant advice. We have revised the sentence as “Our results confirmed the positive correlations of HCY and ED. Meanwhile, B12 was also likely to be significantly associated with ED.” in the “Conclusion” section.