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Risk effects of homocysteine and vitamin B12 in erectile dysfunction discovered in the *FAMHES* project

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Abstract

Objectives: To examine the association between homocysteine (HCY), vitamin B12, folic acid (FA) and erectile dysfunction (ED), the study is conducted.

Design: Based on the Fangchenggang Area Male Health and Examination Survey (FAMHES) project launched from September to December 2009, 1381 samples are included in analyses. ED is evaluated by International Index of Erectile Function (IIEF-5). Regression and subgroups analyses are used.

Results: Although no significant association between FA and ED has been discovered, our results still confirmed risk effects of HCY for ED especially among men with unsatisfactory marital status. Interestingly, vitamin B12 is identified to increase ED risk (OR=1.438, 95%CI=1.070-1.933, P=0.016). In multinomial logistic regression, four severity grades of ED were defined. B12 is confirmed to promote mild ED especially (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: OR=1.620, 95%CI=1.141-2.300, P=0.007), among 40-49 years' men (OR=2.907, 95%CI=1.402-6.026, P=0.004). Moreover, along with the increase of B12, the risk effect enhanced. **Conclusions:** In summary, HCY might be the risk factor of ED. And B12 is significantly associated with ED development. As for the exact effects of B12 for ED, further studies were needed, which might pave the way for the treatment of B12 in ED furtherly.

Keywords: erectile dysfunction, homocysteine, vitamin B12, folic acid

Strengths and limitations of this study

1. Our study is a cross-sectional study, mainly based on the large Fangchenggang Area Male Health and Examination Survey (FAMHES) project, with 4303 men in total.
2. Comprehensive analyses are involved in this study, including baseline analysis, linear and logistic regression analyses and multinomial logistic regression analysis.
3. Additionally, according to the changes of HCY, B12 and FA levels, and the order of severity of ED, the complex associations between HCY, B12, FA and ED are investigated.
4. Meanwhile, the effects of ages, marital status, and educational status are also taken into full consideration.
5. However, as a cross-sectional analysis, the exact mechanisms of the relationship between HCY, B12, FA and ED cannot be explained definitely.

Introduction

Erectile dysfunction (ED) is defined as the inability to acquire and maintain satisfying sexual intercourse with a sufficient erection, affecting up to 53.4% of men aged 30 to 80 years. [1] After 40 years, the morbidity is increased sharply. [2-3] As a prediction, it is estimated that the cases of ED might reach to 322 million by the year 2025 worldwide. [4] However, the definite pathogenesis is unclear.

Various factors are identified to influence the development of ED, such as smoking, hypertension, hyperlipidaemia etc., among them, the vascular component is dominant. [5-6] Moreover, ED might be one of the markers of cardiovascular disease (CAD). [7] Recently, as one of associated cardiovascular factors, homocysteine (HCY) is also said to be an independent risk factor for ED. [8-9] As the cofactors of HCY, folic acid (FA) was also identified to be associated with ED. [10] However, limited studies had been focused on relevance of the B12 level and ED. In order to investigate the exact association between HCY, B12, FA and ED comprehensively, our study is conducted based on the Fangchenggang Area Male Health and Examination Survey (*FAMHES*) project. This time, our study might pave the way for the treatment of ED on the basis of the balance of HCY, B12 and FA.

Methods and Materials

Population and data collection

FAMHES is a population-based project, which is mainly performed to investigate the environmental and genetic factors, as well as their interrelations. From September to December 2009, 4303 men coming for routine physical examination at the Medical Centre in Fangchenggang First People's Hospital were collected. Then, 3593 participants responded to the further interviews (response rate=83.5%). [11] No distinct differences were detected between the men who participated in the interviews and those who did not. Written informed consents were signed by all participants. This study was approved by the medical ethics committee of Guangxi Medical University.

All participants were asked to provide blood samples between 8:00 and 11:00 in the morning, after fasting for at least 8 h (overnight). Then, these bloods 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. Serum B12 and FA were detected with electrochemiluminescence immunoassay. And serum HCY was measured with enzymatic cycling methods.

A comprehensive questionnaire was also applied in this project. This process was mainly performed by the trained investigators using a standardized protocol, with a face-to-face interview. Essential information (age, sex, smoking, drinking, and so on) and complete physical examinations (height, weight, waistline, hipline, etc.) were collected. Smoking status and alcohol consumption were defined as Yes or No. The marital status was classified into live together (married or cohabitation without marriage) and alone (spinsterhood or widowed). Meanwhile, according to the years of education, three groups could be defined (0-6 years: Primary education; 7-12 years: Intermediate education; ≥13 years: Superior education). In the physical examination, body weight with thin clothing and height without shoes were measured. Then, body mass index

(BMI) was calculated with the formula of weight/(height)^2. The waist circumference was measured at the midpoint between the inferior costal margin and the superior iliac crest on the midaxillary line. The hipline was defined as the maximum circumference over the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference/hipline.

Patient and Public Involvement

Patients and public were not involved in the development of the research question, design, and recruitment of this study.

ED definition and grouping

In this study, the International Index of Erectile Function (IIEF-5) was applied to define the ED. [12] The IIEF-5 system has five questions, which mainly covers the conditions of erection confidence, erection firmness, maintenance ability, maintenance frequency, and satisfaction, with the scores ranging from 5 to 25. Each question has six selections. According to the orders of answers, the scores are defined as 0-5. Then, participants can be divided into ED (IIEF-5≤21) and Non-ED (IIEF-5>21). According to the symptoms, ED can also be classified into five groups: none (IIEF-5 score 22–25); mild (17–21); moderate (12–16); and severe symptom (5–11). [11, 13] In addition, HCY level can also be divided into Normal (the level of HCY was 5-15μmol/L) and hyperhomocysteinemia (when HCY >15μmol/L). [14]

Participants screening

In order to acquire the eligible participants for this study, we developed rigorous exclusion criteria: (i) without complete data for the individual information and IIEF-5 score; (ii) without complete data for HCY, B12 and FA, or refused to provide the blood samples; (iii) with diseases such as cardiovascular diseases, inflammatory/immune diseases and kinds of cancers, etc., which might influence the level of HCY, B12 and FA; (iv) similarly, currently taking drugs which could affect the HCY, B12 and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine, glucocorticoids, or other steroidal drugs. Then, 1381 samples were included for the further analyses.

Statistical analysis

Before analysis, all the variates were tested for Gaussian distribution. Then, HCY, B12 and FA were logarithmically transformed, in order to ensure the approximate Gaussian distribution. Based on the 22 scores of IIEF-5, two groups were defined (ED and Non-ED). And Student's t-test and the X^2 test were applied in the baseline analysis. Then, linear and logistic regression analyses were used, with the IIEF-5 scores and binary variable (ED or Non-ED) as the dependent factors respectively. Three adjusted models were used: Unadjusted, Age-adjusted and Multivariate adjusted models. In the Multivariate adjusted model, the covariates were as follows: age, smoking status, alcohol consumption, BMI and WHR. Then, the multinomial logistic regression analysis was also performed to discover the potential association between HCY, B12, FA and ED furtherly, along with the order of severity of ED or the changes of HCY, B12 and FA levels quartile (Q1<levels of 25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%). Additionally, considering the non-negligible influences of ages in the ED risk, we also grouped the participants on the basis of ages (<40, 40-49, 50-59 and ≥60 years). Additionally, according to the groups of marital status, and educational

status, the logistic regression analyses were also conducted. All statistical tests were two-tailed, which were performed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

Results

In the baseline analysis, based on IIEF-5, ED and Non-ED groups were defined. In line with previous study, the age of ED (37.99±10.75, years) is older than Non-ED group (34.18±8.47, years, P<0.001). Meanwhile, B12 level is significantly higher in ED (P=0.015). Although, no significant difference is shown for HCY level, the proportion of hyperhomocysteinemia is higher in the ED (43.02%) than Non-ED (37.52%, P=0.037). In addition, the proportion of alcohol consumption (P=0.032), and educational status (P<0.001) are also identified to be statistic difference in two groups. (**Table 1**)

Signal for the association between HCY and ED

Although after comprehensive analyses, no significant association between HCY level and ED is discovered. (**Table 2-4**) When grouping the samples according to the age, we find that the HCY might be associated with ED especially in the old men (age≥60). (**Table S1**) The similar relevance was confirmed in the marital status (alone, Unadjusted severe ED: OR=4.385, 95%CI=1.070-17.974, P=0.040; Age-adjusted severe ED: OR=5.085, 95%CI=1.195-21.636, P=0.028), which suggests that the elevated HCY level can increase the risk of ED. (**Table S2**)

Then, the HCY is divided into Normal (the level of HCY was 5-15μmol/L) and hyperhomocysteinemia (when HCY >15μmol/L). Similarly, the risk effects of HCY seem to be more prominent in the unsatisfactory marital status (alone, severe ED, age-adjusted: OR=2.448, 95%CI=1.046-5.733, P=0.039). (**Table S2**)

B12 level significantly associated with erectile dysfunction

In the process of investigating the association between ED and B12, linear and logistic regression analyses are also applied. Although with three adjusted models, no significant results are detected for B12 in linear regression analysis. As for the binary logistic regression, B12 is identified to be a risk factor of ED in the Unadjusted model (OR=1.438, 95%CI=1.070-1.933, P=0.016). However, the association is disappeared in other adjusted models. (**Table 2**) Furtherly, we try to discover the relationship between B12 and ED, based on the severity grades of ED. Interestingly, B12 is confirmed to be the risk factor for 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 3**) Then, the level of B12 is divided into quartiles. The result suggests that along with the increases of B12 level, the risk effect for ED enhance (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 4**)

As shown in the analyses above, after adjusting for age the significant association between B12 and ED disappears (**Table 2** and **Table 4**), which suggests that the age cannot be ignored in investigating the effect of B12 in ED. So, we group the participants into four parts (ages <40, 40-49, 50-59 and ≥60 years). Our results show that the risk effect of B12 for 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 S1**)

Our baseline analysis discovers different proportions of educational status in ED and non-ED. In order to discuss the influences of marital and educational status in the relations of B12 and ED, we performed the further subgroup analyses. Similar to previous results, B12 are also identified to be associated with mild ED, even after multivariate adjustment (marital status, live together: OR=1.501, 95%CI=1.035-2.175, P=0.032; alone: OR=3.449, 95%CI=1.113-10.692, P=0.032; educational status, Intermediate: OR=1.858, 95%CI=1.214-2.845, P=0.004). (**Table S2**)

Discussion

ED is one of common diseases, affecting a large number of male populations.[1-4] Recent studies suggest HCY may be an independent risk factor for ED. [8-9] In order to test this association, the study is conducted based on larger population-based *FAMHES* project. After analysis, HCY is detected to increase the risk of ED, especially for the severe ED. Moreover, B12 may also be the risk factor for mild ED. However, no significant association between FA and ED is discovered in our study.

HCY is a thiol-containing amino acid, mainly from methionine. In the process of transformation, two steps are needed. Firstly, methionine is catalyzed to be S-adenosyl methionine (SAM) by the enzyme SAM synthase. As a major methyl group donor for various methylation reactions, SAM is mainly transformed into S-adenosylhomocysteine (SAH) after loss of the methyl group. At last, SAH is hydrolyzed by SAH hydrolase to form HCY and adenosine. HCY is involved in two pathway, including remethylation (RM) and transsulfuration (TS). In the RM pathway, HCY can regenerates methionine with methylenetetrahydrofolate reductase (MTHFR), in which the FA and vitamin B12 act as cofactors. As for TS pathway, HCY is catalyzed by cystathione-β-synthase (CBS) and γ-cystathionase. [15-16] HCY is said to be associated with many diseases and health conditions, such as psychological disorders, [17-18] lipid profiles, [19] renal Impairment [20] and Inflammatory/Immune factors [21] etc. Additionally, HCY is also identified to be a useful marker for the cardiovascular diseases. [22-23] Meanwhile, studies had suggested that ED might share the common risk factors of cardiovascular diseases. And it could be a potentially predictive factor for cardiovascular and other chronic diseases. [24] Based on this relevance, it is said that HCY might be a risk factor of ED in some extent. [8-9] In order to make it clear, our study was conducted. As expected, we identified that HCY could increase the risk of ED especially for severe ED. The main mechanism might be that the HCY could influence endothelial dysfunction and nitric oxide (NO) diffuses. Previously, in vitro and in vivo studies discovered that HCY could be a toxin for the vasculature by inducing endothelial dysfunction. [25] Additionally, NO mainly participates in the vascular dilation, smooth muscle relaxation (including genitourinary smooth muscle), and permitting penile erection. [26-27] Studies provided that the increased HCY could inhibit NO-synthase (which could influence the production of NO), then influencing the development of ED. [28] So, on the basis of these processes, we could understand the risk effect of HCY in inducing ED. Additionally, unsatisfactory marital status would also influence this association, which hinted the pathogenesis of psychological factors for ED in some extent.

B12 is also known as cobalamin. Similar to FA, they are important co-factor in the Methionine synthesis and homocysteine metabolism. [29] Although pervious study had identified that the FA might be a potential protective factor for ED, [30] no significant association had been detected this time. As for B12, opposite to HCY, it was said to protect from the ED. [31] However, in our study, B12

is identified as a risk factor of mild ED. In addition, along with the deterioration of ED, the risk effect is decreased. (**Table 3**) Meanwhile, high level of B12 can promote the development of ED. (**Table 4**) There are two explains. Firstly, our results suggest that the function of B12 in ED might be dose-dependent. Excess B12 level would increase the risk of mild ED with some unclear mechanisms. Secondly, increased B12 might be negative feedback of ED patients for this disease. At the beginning of the disease, defense mechanism is triggered. As a potential protective factor, the absorption of B12 is enhanced. Combining with the limited studies, our study can also propose that B12 is significantly associated with ED development. As for the exact effects of B12 for ED, further studies were needed, which might pave the way for the treatment of B12 in ED furtherly.

Limitations

In one hand, our study verified the previous conclusions that the HCY could increase the risk of ED. On the other hand, the function of B12 on ED is one interesting discussion. There were some limitations needed to be noticed: (i) this study is a cross-sectional analysis, which just reflects the status of specific time point and populations; (ii) there are limited numbers of samples with the primary educational status. So, the results are exaggerated, which is needed to be examined further; (iii) although we have identified significant association between B12 and ED, the exact mechanism and effects were unclear until now.

Conclusions

ED is one of common male diseases. In order to discover the functions of HCY, B12 and FA in ED, this study was conducted. Our results confirmed the pathogenic effect of HCY for ED, especially for severe ED. Meanwhile, B12 was also significantly associated with ED. Further studies should be focused on the potential mechanisms and therapeutic effect of B12 in ED.

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Conflict of Interest

There are no conflicts of interests.

Author Contributions

Y.C., J.L., Z.N.M., and J.W.C. participated in sample collection, field investigation, design, writing and modification of all the paper. Y.C. and J.L. took part in the statistical analysis. Z.N.M. and J.W.C. provided important advices for this paper.

Data Sharing Statement

The data for this study was available in the supplementary materials. Further questions could be

sent to ZN.M (zengnanmo@hotmail.com) and JW.C (chengjiwen1977@foxmail.com).

References

1. Braun M, Wassmer G, Klotz T, et al. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res 2000;12:305-11. [PubMed: 11416833]

2. Pinnock CB, Stapleton AM, Marshall VR. Erectile dysfunction in the community: a prevalence study. Med J Aust 1999;171:353-7. [PubMed: 10590723]

3. Nicolosi A, Moreira ED Jr, Shirai M, et al. Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectile dysfunction. Urology 2003;61:201-6. [PubMed: 10590723]

4. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 1999;84:50-56. [PubMed: 10444124]

5. Kloner RA, Speakman M. Erectile dysfunction and atherosclerosis. Curr Atheroscler Rep 2002;4:397-401. [PubMed: 12162940]

6. Sullivan ME, Keoghane SR, Miller MA. Vascular risk factors and erectile dysfunction. BJU Int 2001;87:838-45. [PubMed: 11412223]

7. Nehra A, Jackson G, Miner M, et al. Diagnosis and treatment of erectile dysfunction for reduction of cardiovascular risk. J Urol 2013;189:2031-8. [PubMed: 23313195]

8. 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. [PubMed: 10510122]

9. 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. [PubMed: 27709655]

10. Sansone M, Sansone A, Romano M, et al. Folate: a possible role in erectile dysfunction? Aging Male. 2017 Nov 20:1-5. [PubMed: 29157083]

11. 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. [PubMed: 25343742]

12. Rosen RC, Cappelleri JC, Smith MD, et al. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res 1999;11:319-26. [PubMed: 10637462]

13. Kupelian V, Araujo AB, Chiu GR, et al. Relative contributions of modifiable risk factors to erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. Prev Med 2010;50:19-25. [PubMed: 19944117]

14. Guo H, Chi J, Xing Y, et al. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res 2009; 129:279-84. [PubMed: 19491420]

15. Long Y, Nie J. Homocysteine in Renal Injury. Kidney Dis (Basel) 2016; 2:80-7. [PubMed: 27536696]

16. Lai WK, Kan MY. Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1-12. [PubMed: 26201664]

17. Salagre E, Vizuete AF, Leite M, et al. Homocysteine as a peripheral biomarker in bipolar

disorder: A meta-analysis. Eur Psychiatry. 2017;43:81-91. [PubMed: 28371745]
18. Elstgeest LE, Brouwer IA, Penninx BW, et al. Vitamin B12, homocysteine and depressive symptoms: a longitudinal study among older adults. Eur J Clin Nutr 2017;71:468-75. [PubMed: 28145420]
19. Momin M, Jia J, Fan F, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis 2017; 16:54. [PubMed: 28288621]
20. Chen J, Li G, Xu Z, et al. Elevated Plasma Homocysteine Level Increased the Risk of Early Renal Impairment in Acute Ischemic Stroke Patients. Cell Mol Neurobiol 2017;37:1399-1405. [PubMed: 28275883]
21. Li T, Chen Y, Li J, et al. Serum Homocysteine Concentration Is Significantly Associated with Inflammatory/Immune Factors. PLoS One 2015; 10:e0138099. [PubMed: 26367537]
22. Sahu A, Gupta T, Kavishwar A, et al. Cardiovascular Diseases Risk Prediction by Homocysteine in Comparison to other Markers: A Study from Madhya Pradesh. J Assoc Physicians India 2015;63:37-40. [PubMed: 27608690]
23. Yeh JK, Chen CC, Hsieh MJ, et al. Impact of Homocysteine Level on Long-term Cardiovascular Outcomes in Patients after Coronary Artery Stenting. J Atheroscler Thromb 2017;24:696-705. [PubMed: 27803490]
24. Baumann F, Hehli D, Makaloski V, et al. Erectile dysfunction - overview from a cardiovascular perspective. Vasa 2017; 1-7. [PubMed: 28486869]
25. McDowell IF, Lang D. Homocysteine and endothelial dysfuncton: A link with cardiovascular disease. J Nutr 2000;130: 369S-372S. [PubMed: 10721909]
26. Rajfer J, Aronson WJ, Bush PA, et al. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Engl J Med 1992;326:90-94. [PubMed: 1309211]
27. Deanfield J, Donald A, Ferri C, et al. Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension: Endothelial function and dysfunction. Part I: methodological issues for assessment in the different vascular beds: a statement by the working group on endothelin and endothelial factors of the European society of hypertension. J Hypertens 2005;23:7-17. [PubMed: 15643116]
28. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-375. [PubMed: 10475890]
29. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2:299-316. [PubMed: 22254022]
30. Yan WJ, Yu N, Yin TL, et al. A new potential risk factor in patients with erectile dysfunction and premature ejaculation: folate deficiency. Asian J Androl 2014;16:902-906. [PubMed: 25080932]
31. Giovannone R, Busetto GM, Antonini G, et al. Hyperhomocysteinemia as an Early Predictor of Erectile Dysfunction: International Index of Erectile Function (IIEF) and Penile Doppler Ultrasound Correlation With Plasma Levels of Homocysteine. Medicine (Baltimore) 2015;94:e1556. [PubMed: 26426624]

	ED	Non-ED	P
No.	688	693	
Age, years	**37.99±10.75**	**34.18±8.47**	**<0.001**
BMI, Kg/m^2	23.27±3.26	23.37±3.48	0.591
WHR	0.88±0.06	0.88±0.06	0.253
HCY, μmol/L	14.97±4.11	15.34±11.09	0.524
Normal HCY	392 (56.98%)	433 (62.48%)	
Hyperhomocysteinemia	**296 (43.02%)**	**260 (37.52%)**	**0.037**
B12, pg/ml	**718.53±234.37**	**688.74±229.68**	**0.015**
FA, ng/ml	9.56±2.72	9.89±11.28	0.594
Smoke			
Yes	392 (56.98%)	385 (55.56%)	
No	296 (43.02%)	308 (44.44%)	0.594
Drink			
Yes	**586 (85.17%)**	**617 (89.03%)**	
No	**102 (14.83%)**	**76 (10.97%)**	**0.032**
Marital status			
Live together	595 (86.48%)	578 (83.41%)	
Alone	93 (13.52%)	115 (16.59%)	0.110
educational status[a]			
Primary	**19 (2.76%)**	**5 (0.72%)**	
Intermediate	**488 (70.93%)**	**411 (59.39%)**	
Superior	**181 (26.31%)**	**276 (39.89%)**	**<0.001**

Table 1. The characteristics of the eligible samples in the analysis

a. One participant without the information of educational status in the Non-ED group

* Normal HCY: 5-15μmol/L; hyperhomocysteinemia: >15umol/L

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

	Unadjusted			Age-adjusted			Multivariate adjusted		
	BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P
IIEF-5									
HCY	-0.202	-1.080, 0.676	0.651	0.139	-0.732, 1.009	0.755	0.084	-0.787, 0.956	0.850
Binary HCY	-0.338	-0.817, 0.142	0.167	-0.146	-0.622, 0.330	0.548	-0.186	-0.663, 0.291	0.444
B12	0.048	-0.600, 0.696	0.885	0.212	-0.428, 0.852	0.515	0.404	-0.259, 1.068	0.232
FA	0.112	-0.668, 0.891	0.779	0.388	-0.384, 1.160	0.986	0.324	-0.496, 1.145	0.438
Binary									
HCY	1.137	0.766-1.687	0.524	0.959	0.641-1.435	0.839	0.986	0.658-1.479	0.986
Binary HCY	**1.258**	**1.014-1.560**	**0.037**	1.151	0.923-1.435	0.212	1.174	0.940-1.466	0.157
B12	**1.438**	**1.070-1.933**	**0.016**	1.338	0.992-1.805	0.057	1.311	0.961-1.788	0.087
FA	1.100	0.775-1.561	0.594	0.956	0.668-1.367	0.804	1.041	0.710-1.527	0.835

Table 2. The linear and binary regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

	HCY			Binary_HCY			B12			FA		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
ED-Unadjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	1.081	0.695-1.681	0.730	1.246	0.980-1.583	0.072	**1.694**	**1.207-2.376**	**0.002**	1.180	0.801-1.740	0.402
Moderate (12–16)	1.258	0.649-2.439	0.497	1.298	0.896-1.880	0.168	1.187	0.715-1.972	0.508	0.960	0.519-1.776	0.896
Severe (5–11)	1.259	0.562-2.820	0.575	1.258	0.799-1.980	0.322	0.891	0.496-1.599	0.698	0.923	0.434-1.963	0.834
ED-age-adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.948	0.607-1.480	0.814	1.166	0.914-1.486	0.217	**1.596**	**1.135-2.244**	**0.007**	1.052	0.710-1.558	0.800
Moderate (12–16)	0.929	0.455-1.898	0.840	1.095	0.747-1.605	0.643	1.409	0.635-1.733	0.851	0.762	0.404-1.437	0.401
Severe (5–11)	1.068	0.467-2.443	0.876	1.150	0.726-1.820	0.552	0.839	0.471-1.495	0.551	0.794	0.370-1.705	0.554
ED- Multivariate adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.973	0.621-1.524	0.904	1.188	0.929-1.517	0.169	**1.620**	**1.141-2.300**	**0.007**	1.184	0.775-1.808	0.435
Moderate (12–16)	0.965	0.472-1.971	0.922	1.119	0.762-1.643	0.567	0.972	0.576-1.640	0.915	0.777	0.401-1.507	0.456
Severe (5–11)	1.132	0.491-2.613	0.771	1.187	0.748-1.885	0.467	0.717	0.388-1.324	0.288	0.821	0.368-1.831	0.631

Table 3. Multinomial logistic regression for the association between ED and HCY, B12 and FA

* The symptoms of ED were divided into None (IIEF-5= 22–25), Mild (17–21), Moderate (12–16) and Severe (5–11). And the None (22-25) was the reference.

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

	Unadjusted			Age-adjusted			Multivariate adjusted		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.130	0.838-1.525	0.422	1.022	0.753-1.387	0.887	1.012	0.744-1.377	0.938
Q3	1.292	0.958-1.743	0.094	1.187	0.875-1.610	0.271	1.210	0.890-1.646	0.224
Q4	1.276	0.946-1.720	0.110	1.091	0.803-1.483	0.578	1.103	0.810-1.502	0.534
B12									
Q1	1	1	1	1	1	1	1	1	1
Q2	0.917	0.680-1.236	0.569	0.893	0.659-1.209	0.464	0.899	0.662-1.221	0.496
Q3	0.988	0.733-1.333	0.939	0.972	0.717-1.316	0.853	0.986	0.726-1.338	0.927
Q4	**1.452**	**1.076-1.961**	**0.015**	1.299	0.955-1.765	0.095	1.286	0.945-1.752	0.110
FA									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.313	0.974-1.770	0.074	1.325	0.978-1.795	0.069	1.332	0.981-1.811	0.067
Q3	1.300	0.963-1.755	0.086	1.243	0.916-1.687	0.163	1.286	0.944-1.751	0.111
Q4	1.198	0.888-1.616	0.236	1.094	0.806-1.487	0.564	1.116	0.819-1.522	0.487

Table 4. Association between HCY, B12, FA and ED along with the increased levels of these indexes

* Multinomial logistic regression was applied.

* The levels of HCY, B12, and FA was divided into quartile (Q1<levels of 25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%).

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

		No. ED	No. Non-ED	HCY			Binary HCY			B12			FA		
				BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P
Ages <40															
IIEF-5		409	531	-0.246	-1.32, 0.837	0.656	-0.269	-0.845, 0.307	0.359	0.627	-0.202, 1.456	0.138	-0.137	-1.135, 0.862	0.788
ED				0.987	0.594-1.639	0.960	1.147	0.876-1.501	0.319	1.207	0.818-1.780	0.344	1.146	0.718-1.829	0.567
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.961	0.547-1.688	0.889	1.185	0.881-1.594	0.263	1.460	0.945-2.255	0.088	1.162	0.692-1.950	0.571
	Moderate			0.794	0.282-2.238	0.662	0.843	0.486-1.462	0.544	0.957	0.444-2.066	0.912	1.015	0.401-2.568	0.974
	Severe			1.429	0.469-4.357	0.530	1.351	0.732-2.495	0.336	0.544	0.223-1.326	0.180	1.276	0.432-3.766	0.659
40-49		176	131												
IIEF-5				0.501	-1.025, 2.207	0.519	-0.109	-1.060, 0.841	0.821	0.320	-0.904, 1.544	0.607	0.990	-0.622, 2.602	0.228
ED				0.842	0.398,-1.778	0.651	1.129	0.710-1.795	0.608	1.692	0.925-3.094	0.088	0.878	0.398-1.937	0.747
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.820	0.358-1.878	0.638	1.135	0.685-1.882	0.623	**2.907**	**1.402-6.026**	**0.004**	1.174	0.495-2.785	0.716
	Moderate			0.951	0.288-3.146	0.935	1.267	0.598-2.682	0.537	0.550	0.224-1.355	0.194	0.380	0.102-1.407	0.147
	Severe			0.845	0.140-5.123	0.855	0.905	0.318-2.578	0.852	0.910	0.243-3.403	0.889	0.579	0.105-3.183	0.530
50-59		69	21												
IIEF-5				4.297	-0.514, 9.108	0.079	1.597	-0.408, 3.602	0.117	-2.046	-4.862, 0.770	0.152	-1.649	-5.387, 2.088	0.383
ED				0.314	0.028-3.584	0.351	0.867	0.309-2.430	0.786	1.521	0.355-6.516	0.572	1.773	0.265-11.867	0.555
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.379	0.024-5.901	0.489	0.927	0.299-2.875	0.895	0.859	0.171-4.308	0.853	1.601	0.198-12.945	0.659
	Moderate			0.669	0.030-14.774	0.799	1.326	0.356-4.931	0.674	4.335	0.603-31.187	0.145	1.042	0.086-12.647	0.974
	Severe			0.046	0.001-1.941	0.107	0.358	0.077-1.660	0.189	2.060	0.256-16.573	0.497	3.751	0.263-53.516	0.330
≥60		34	10												
IIEF-5				-0.479	-7.400, 6.442	0.889	-0.116	-3.491, 3.259	0.945	-0.526	-4.308, 3.255	0.779	2.899	-2.726, 8.523	0.303
ED				29.170	0.379-2.243E3	0.128	2.544	0.409-15.822	0.317	0.714	0.089-5.723	0.751	0.523	0.018-15.079	0.705
	None			1	1	1	1	1	1	1	1	1	1	1	1

Mild	**767.519**	**1.649-3.573E5**	**0.034**	4.093	0.337-49.760	0.269	0.677	0.038-12.139	0.791	0.645	0.008-52.675	0.845
Moderate	11.236	0.098-1.289E3	0.317	2.266	0.283-18.119	0.441	1.067	0.092-12.408	0.959	2.717	0.049-150.689	0.626
Severe	70.920	0.291-1.729E4	0.129	3.281	0.316-34.092	0.320	0.424	0.037-4.866	0.491	0.027	0.000-3.577	0.148

Table S1. Association between ED and HCY, B12, FA level in four ages groups (<40, 40-49, 50-59 and ≥60 years) after adjusting multivariate

* Linear regression for the IIEF-5; binary regression analysis for ED; multinomial logistic regression for the severity of symptom of ED

* ED is divided into four groups according to the IIEF-5 scores: None=22-25 scores, Mild=17-21 scores, Moderate=12-16 scores and Severe=5-11 scores. None ED is treated as the reference.

* ED: erectile dysfunction; HCY= homocysteine; B12= vitamin B12; FA= folic acid; BMI: body mass index; WHR: waist hip rate; OR: odd ratio; 95%CI: 95% confidence interval

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* Multi-adjusted: age, BMI, WHR, smoke and drink

	ED grading	HCY			Binary_HCY			B12			FA		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Unadjusted													
Marital status													
Live together		1.070	0.698-1.640	0.757	1.237	0.979-1.562	0.074	**1.400**	**1.010-1.940**	**0.044**	1.283	0.860-1.916	0.222
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.077	0.673-1.722	0.758	1.239	0.959-1.601	0.101	**1.484**	**1.033-2.132**	**0.033**	1.295	0.833-2.012	0.251
	Moderate	1.267	0.629-2.555	0.508	1.367	0.920-2.032	0.122	1.105	0.636-1.921	0.723	1.158	0.583-2.303	0.675
	Severe	0.678	0.227-2.027	0.487	0.987	0.566-1.722	0.964	1.534	0.697-3.375	0.288	1.492	0.580-3.841	0.407
Alone		1.523	0.540-4.299	0.426	1.346	0.765-2.370	0.303	1.458	0.705-3.013	0.309	0.577	0.246-1.356	0.207
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.813	0.207-3.194	0.767	1.169	0.580-2.355	0.662	**3.950**	**1.331-11.719**	**0.013**	0.750	0.285-1.976	0.561
	Moderate	0.985	0.128-7.560	0.988	0.812	0.267-2.470	0.714	1.568	0.352-6.975	0.555	0.330	0.058-1.878	0.211
	Severe	**4.385**	**1.070-17.974**	**0.040**	2.249	0.974-5.193	0.058	0.605	0.252-1.450	0.260	0.460	0.116-1.824	0.269
educational status													
Primary		1.586	0.018-142.652	0.841	0.875	0.116-6.579	0.897	5.169	0.188-142.118	0.331	0.399	0.095-1.676	0.209
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	3.257	0.018-596.353	0.657	0.500	0.045-5.514	0.571	4.007	0.136-117.975	0.421	0.455	0.088-2.364	0.349
	Moderate	0.489	0.002-114.662	0.797	1.500	0.136-16.542	0.741	**1.266E^{3}**	**2.303-6.962E^{5}**	**0.026**	0.423	0.060-2.975	0.387
	Severe	2.199	0.007-686.953	0.788	1.000	0.080-12.557	1.000	2.627	0.200-34.591	0.463	0.205	0.009-4.472	0.314
Intermediate		1.667	0.989-2.811	0.055	1.305	0.993-1.716	0.056	**1.513**	**1.049-2.182**	**0.027**	1.187	0.747-1.887	0.469
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.571	0.883-2.795	0.124	1.295	0.958-1.752	0.093	**1.838**	**1.215-2.781**	**0.004**	1.304	0.779-2.181	0.312
	Moderate	1.619	0.692-3.785	0.266	1.265	0.806-1.986	0.307	1.132	0.620-2.067	0.687	0.888	0.411-1.919	0.763
	Severe	2.355	0.876-6.326	0.089	1.429	0.829-2.463	0.199	0.934	0.452-1.926	0.852	1.160	0.451-2.980	0.759
Superior		0.874	0.449-1.699	0.691	1.436	0.986-2.093	0.060	0.938	0.537-1.640	0.823	1.117	0.586-2.129	0.738
	None	1	1	1	1	1	1	1	1	1	1	1	1

	Mild	0.765	0.351-1.669	0.501	1.403	0.924-2.130	0.112	1.156	0.612-2.183	0.655	1.202	0.587-2.462	0.615
	Moderate	1.581	0.567-4.414	0.381	1.800	0.849-3.818	0.126	0.640	0.225-1.821	0.403	1.244	0.345-4.481	0.739
	Severe	0.479	0.064-3.567	0.472	1.170	0.461-2.970	0.741	0.491	0.142-1.691	0.259	0.569	0.116-2.788	0.487
Age-Adjusted													
Marital status													
Live together		9.868	0.559-1.347	0.528	1.110	0.873-1.412	0.394	1.333	0.954-1.861	0.092	1.122	0.743-1.694	0.584
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.940	0.586-1.508	0.796	1.157	0.892-1.501	0.271	1.436	0.996-2.072	0.053	1.176	0.752-1.839	0.478
	Moderate	0.874	0.402-1.902	0.735	1.110	0.735-1.676	0.620	1.015	0.583-1.766	0.958	0.947	0.469-1.909	0.878
	Severe	0.378	0.114-1.258	0.113	0.762	0.428-1.357	0.356	1.353	0.622-2.943	0.445	1.193	0.459-3.097	0.717
Alone		1.611	0.566-4.586	0.371	1.406	0.793-2.493	0.243	1.391	0.653-2.961	0.392	0.622	0.257-1.506	0.292
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.216-3.352	0.817	1.210	0.598-2.451	0.596	**3.742**	**1.258-11.129**	**0.018**	0.799	0.294-2.169	0.659
	Moderate	1.019	0.132-7.836	0.986	0.831	0.272-2.542	0.745	1.492	0.339-6.562	0.597	0.341	0.060-1.947	0.226
	Severe	**5.085**	**1.195-21.636**	**0.028**	**2.448**	**1.046-5.733**	**0.039**	0.450	0.175-1.159	0.098	0.516	0.122-2.174	0.367
educational status													
Primary		0.285	0.001-61.236	0.647	0.212	0.010-4.431	0.317	10.044	0.027-3.674E^{3}	0.444	0.421	0.080-2.228	0.309
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.588	0.004-601.127	0.879	0.228	0.010-5.326	0.357	4.021	0.062-259.697	0.513	0.485	0.093-2.542	0.392
	Moderate	0.001	7.773E^{-8}-6.096	0.116	0.139	0.003-6.581	0.316	3.874E^{3}	1.164-1.289E^{7}	0.046	0.426	0.024-7.587	0.561
	Severe	0.035	1.430E^{-5}-84.089	0.398	0.118	0.002-6.145	0.290	8.466	0.008-8.474E^{3}	0.545	0.162	0.005-5.514	0.311
Intermediate		1.392	0.820-2.365	0.221	1.209	0.915-1.597	0.182	1.384	0.954-2.009	0.087	1.073	0.669-1.722	0.769
	None	1	1	1	1	1		1	1	1	1	1	1
	Mild	1.382	0.774-2.468	0.275	1.229	0.906-1.668	0.186	**1.723**	**1.133-2.620**	**0.011**	1.206	0.717-2.030	0.480
	Moderate	1.145	0.470-2.787	0.766	1.084	0.682-1.724	0.733	0.966	0.529-1.764	0.911	0.754	0.346-1.643	0.478
	Severe	1.915	0.698-5.257	0.207	1.298	0.748-2.254	0.354	0.840	0.409-1.726	0.635	1.022	0.397-2.632	0.964
Superior		0.779	0.391-1.552	0.477	1.324	0.900-1.948	0.154	0.953	0.540-1.684	0.869	0.898	0.461-1.750	0.751

	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.672	0.297-1.521	0.340	1.289	0.841-1.976	0.245	1.175	0.618-2.238	0.623	0.960	0.458-2.012	0.914
	Moderate	1.421	0.486-4.155	0.521	1.583	0.736-3.405	0.240	0.682	0.241-1.926	0.470	0.917	0.244-3.440	0.898
	Severe	0.521	0.074-3.647	0.511	1.202	0.472-3.061	0.699	0.473	0.132-1.690	0.249	0.622	0.125-3.096	0.562
Multivariate adjusted													
Marital status													
Live together		0.894	0.575-1.390	0.619	1.137	0.893-1.448	0.299	1.349	0.962-1.891	0.083	1.176	0.773-1.789	0.449
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.963	0.598-1.548	0.875	1.181	0.909-1.534	0.213	**1.501**	**1.035-2.175**	**0.032**	1.245	0.790-1.961	0.345
	Moderate	0.913	0.420-1.985	0.818	1.136	0.751-1.719	0.547	0.971	0.554-1.701	0.918	0.933	0.455-1.915	0.851
	Severe	0.417	0.126-1.378	0.152	0.812	0.454-1.453	0.483	1.227	0.562-2.675	0.608	1.317	0.494-3.514	0.582
Alone		1.602	0.546-4.698	0.390	1.399	0.779-2.512	0.261	1.288	0.558-2.975	0.553	0.678	0.253-1.820	0.441
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.206-3.498	0.821	1.199	0.579-2.481	0.625	**3.449**	**1.113-10.692**	**0.032**	0.959	0.279-3.292	0.947
	Moderate	1.130	0.135-9.481	0.910	0.856	0.276-2.654	0.788	1.244	0.261-5.929	0.784	0.336	0.054-2.069	0.239
	Severe	4.181	0.951-19.374	0.058	2.356	0.989-5.616	0.053	0.302	0.084-1.083	0.066	0.598	0.139-2.570	0.490
educational status													
Primary		0.045	0.000-51.900	0.388	0.098	0.002-5.651	0.262	6.248	0.006-6.418E3	0.605	2.823	0.032-247.907	0.649
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.198	$4.915E^{-5}$-794.766	0.702	0.224	0.004-13.29	0.473	0.001	3.247E-10-3.819E3	0.376	1.806	0.007-479.698	0.836
	Moderate	$2.184E^{-12}$	$2.988E^{-28}$-$1.597E^{4}$	0.150	0.004	1.429E-6-8.838	0.157	**NA**	**NA**	**0.043**	3.396	$0.006\text{-}1.820E^{3}$	0.703
	Severe	1.972E-9	$1.318E^{-24}$-$2.952E^{6}$	0.261	0.008	$4.142E^{-6}$-15.343	0.210	**$8.065E^{-217}$**	**0.000-0.203**	**0.049**	0.516	0.001-457.324	0.849
Intermediate		1.431	0.842-2.432	0.186	1.223	0.925-1.618	0.158	1.428	0.981-2.081	0.063	1.092	0.677-1.764	0.717
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.401	0.782-2.510	0.257	1.238	0.910-1.682	0.174	**1.858**	**1.214-2.845**	**0.004**	1.268	0.748-2.149	0.378
	Moderate	1.225	0.502-2.990	0.656	1.105	0.694-1.761	0.673	0.954	0.519-1.755	0.880	0.707	0.319-1.568	0.394
	Severe	2.044	0.744-5.615	0.165	1.350	0.776-2.351	0.288	0.754	0.364-1.562	0.448	0.969	0.367-2.562	0.950

Superior		0.812	0.405-1.627	0.557	1.360	0.920-2.011	0.123	0.977	0.550-1.738	0.938	0.889	0.449-1.762	0.737
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.702	0.308-1.598	0.399	1.325	0.859-2.044	0.204	1.202	0.632-2.287	0.575	0.898	0.419-1.926	0.783
	Moderate	1.437	0.483-4.270	0.514	1.556	0.718-3.370	0.262	0.647	0.217-1.930	0.435	1.056	0.274-4.063	0.937
	Severe	0.598	0.092-3.890	0.591	1.333	0.513-3.459	0.555	0.496	0.123-2.002	0.325	0.715	0.145-3.529	0.681

Table S2. The logistic regression analyses (binary and multinomial) for the association between ED and HCY, B12 and FA in the subgroups of marital status and educational status

* In the educational status, the Primary group only contains 24 samples. So, some results of regression analyses were exaggerated with these limited data

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* ED status: none (IIEF-5 score 22–25); mild (17–21); moderate (12–16); and severe (5–11).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	*4*
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6
		(*b*) Report category boundaries when continuous variables were categorized	5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	7

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study

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Abstract

Objectives: To examine the association between homocysteine (HCY), vitamin B12 (B12), folic acid (FA), and erectile dysfunction (ED).

Design: Cross-sectional study.

Setting: Guangxi, China.

Participants: Participants (N = 1381) completed questionnaires to determine International Index of Erectile Function (IIEF-5) scores, and the values for HCY, B12 and FA were collected from September 2009 to December 2009.

Measures: ED was evaluated by IIEF-5. Regression and between-group analyses were used.

Results: Although no association between FA and ED has been discovered, our results still confirmed significant correlations between HCY and ED, as did other previous studies, especially for men living alone (spinsterhood or widowed). Interestingly, B12 was also identified to be associated with ED (OR = 1.438, 95% CI = 1.070-1.933, P = 0.016). In multinomial logistic regression, four severity grades of ED were defined. B12 was confirmed to be related to 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: OR = 1.620, 95% CI = 1.141-2.300, P = 0.007), especially among 40–49 year old men (OR = 2.907, 95% CI = 1.402-6.026, P = 0.004). Moreover, ED might also be related to high levels of B12.

Conclusions: In summary, HCY and B12 may be significantly associated with ED, especially B12. 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.

Keywords: erectile dysfunction, homocysteine, vitamin B12, folic acid

Strengths and limitations of this study

1. Our study is a cross-sectional study, mainly based on the large Fangchenggang Area Male Health and Examination Survey (FAMHES) project, with 4303 men in total.
2. Comprehensive analyses are involved in this study, including baseline analysis, linear and logistic regression analyses, and multinomial logistic regression analysis.
3. Additionally, according to the changes in the HCY, B12, and FA levels, and the order of severity of ED, the complex associations between HCY, B12, FA, and ED are investigated.
4. Meanwhile, the effects of age, marital status, and educational status are also taken into full consideration.
5. However, as a cross-sectional analysis, the exact mechanisms of the relationship between HCY, B12, FA, and ED cannot be definitely explained.

Introduction

Erectile dysfunction (ED) is defined as the inability to acquire and maintain satisfying sexual intercourse with a sufficient erection, affecting up to 53.4% of men aged 30 to 80 years. [1] After 40 years of age, the morbidity is increased sharply. [2, 3] As a prediction, it is estimated that cases of ED might reach 322 million worldwide by the year 2025. [4]

Various factors have been identified as influences on the development of ED, such as smoking, hypertension, and hyperlipidemia. Among these influences, the vascular component is dominant. [5, 6] Moreover, ED may be one of the markers of cardiovascular disease (CVD). [7] Recently, as one of the associated cardiovascular factors, homocysteine (HCY) is also said to be an independent risk factor for ED. [8, 9] HCY is a thiol-containing amino acid, mainly from methionine. In the process of transformation, two steps are needed. First, methionine is catalyzed to form S-adenosylmethionine (SAM) by the enzyme SAM synthase. As a major methyl group donor for various methylation reactions, SAM is mainly transformed into S-adenosylhomocysteine (SAH) after loss of the methyl group. At last, SAH is hydrolyzed by SAH hydrolase to form HCY and adenosine. HCY is involved in two pathways, including remethylation (RM) and transsulfuration (TS). In the RM pathway, HCY regenerates methionine with methylenetetrahydrofolate reductase (MTHFR) and folic acid (FA) and vitamin B12 (B12) acting as cofactors. As for the TS pathway, HCY is catalyzed by cystathione-β-synthase (CBS) and γ-cystathionase. [10, 11] As are the cofactors of HCY, FA was also identified to be associated with ED. [12] However, limited studies have been focused on relevance of the B12 level to ED. On the basis of previous studies, we hypothesize that there might be a true association between HCY, B12, FA, and ED.

In order to investigate the exact association between HCY, B12, FA, and ED comprehensively, our study is conducted based on the Fangchenggang Area Male Health and Examination Survey (FAMHES) project. This time, our study might pave the way for the treatment of ED on the basis of the balance of HCY, B12, and FA.

Methods and Materials

Population and data collection

FAMHES is a population-based project, which was mainly performed to investigate environmental and genetic factors, as well as their interrelations. 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. Then, 3593 participants responded for further interviews (response rate = 83.5%). [13] No distinct differences were detected between the men who participated in the interviews and those who did not. Written informed consents were signed by all participants. This study was approved by the Medical Ethics Committee of Guangxi Medical University.

All participants were asked to provide blood samples between 8:00 am and 11:00 am, after fasting for at least 8 h (overnight). Then, 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. Serum B12 and FA were detected with electrochemiluminescence immunoassays, while serum

HCY was measured with enzymatic cycling methods.

Then, all the participants were invited to complete a comprehensive questionnaire. This process was performed by the trained investigators using a standardized protocol with a face-to-face interview. Essential information (e.g., age, sex, smoking, and drinking) was collected, and complete physical examinations (e.g., height, weight, waistline, and hipline) were performed. Smoking status and alcohol consumption were defined as Yes or No. The marital status was classified into living together (married or cohabitation without marriage) and alone (spinsterhood or widowed). Meanwhile, according to the years of education, three groups could be defined (0-6 years: Primary education; 7-12 years: Intermediate education; and ≥ 13 years: Superior education). In the physical examination, body weight with thin clothing and height without shoes were measured. Then, body mass index (BMI) was calculated with the formula of weight/(height)^2. The waist circumference was measured at the midpoint between the inferior costal margin and the superior iliac crest in the midaxillary line. The hipline was defined as the maximum circumference over the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference/hipline. The flow of participants' collection is shown in **Figure S1**.

Patient and Public Involvement

Patients and the public were not involved in the development of the research question and design or recruitment of this study.

ED definition and grouping

In this study, the International Index of Erectile Function (IIEF-5) was applied to define ED. [14] The IIEF-5 system has five questions, which mainly cover the conditions of erection confidence, erection firmness, maintenance ability, maintenance frequency, and satisfaction, with the scores ranging from 5 to 25. Each question has six selections. According to the orders of answers, the scores are defined as 0–5. Then, participants can be divided into ED (IIEF-5 ≤ 21) and Non-ED (IIEF-5 > 21) groups. According to the symptoms, ED can also be classified into five groups: none (IIEF-5 score 22-25); mild (17-21); moderate (12-16); and severe symptoms (5-11). [13, 15] In addition, HCY level can also be divided into normal (HCY 5-15μmol/L) and hyperhomocysteinemia (HCY > 15μmol/L). [16]

Participants screening

In order to acquire the eligible participants for this study, we developed rigorous exclusion criteria: (i) incomplete data for the individual information and IIEF-5 score; (ii) incomplete data for HCY, B12, and FA or refused to provide the blood samples; (iii) with diseases such as cardiovascular diseases, inflammatory/immune diseases, and kinds of cancers, which might influence the levels of HCY, B12, and FA (**Table S1**); and (iv) currently taking drugs that might affect the HCY, B12, and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine, or glucocorticoids (**Table S1**). Then, 1381 participants were included for further analyses. The flow for screening the eligible participants is shown in **Figure S2**.

Statistical analysis

Before analysis, HCY, B12, and 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. Based on the 22 IIEF-5 scores, two groups were defined (ED and Non-ED), and Student's t-test and the chi-squared (X^2) test were applied in the baseline analysis. Then, linear and logistic regression analyses were used, with the IIEF-5 scores and binary variable (ED or Non-ED) as the dependent factors, respectively. Three adjusted models were used: Unadjusted, Age-adjusted, and Multivariate adjusted. In the Multivariate adjusted model, the covariates were as follows: age, smoking status, alcohol consumption, BMI, and WHR. Then, the multinomial logistic regression analysis was also performed to discover the potential association between HCY, B12, FA, and ED, along with the order of severity of ED or the changes in the HCY, B12, and FA levels quartile (Q1 < 25%, 25% ≤ Q2 ≤ 50%, 50% < Q3 ≤ 75%, and Q4 > 75%). Additionally, considering the non-negligible influences of age on the risk of ED, we also grouped the participants on the basis of age (< 40, 40-49, 50-59, and ≥ 60 years old). Additionally, according to the groups of marital status and educational status, the logistic regression analyses were also conducted. All statistical tests were two-tailed, which were performed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The threshold for significance was $P < 0.05$.

Results

In the baseline analysis, based on IIEF-5, the ED and Non-ED groups were defined. In line with previous studies, the age of the ED group (37.99 ± 10.75 years) was older than the Non-ED group (34.18 ± 8.47 years, $P < 0.001$). Meanwhile, B12 levels were significantly higher in the ED group ($P = 0.015$). Although, no significant difference was shown for HCY levels, the proportion of hyperhomocysteinemia was higher in the ED group (43.02%) than in the Non-ED group (37.52%, $P = 0.037$). In addition, the proportion of alcohol consumption ($P = 0.032$) and educational status ($P < 0.001$) were also identified to be statistically significantly different in the two groups (**Table 1**).

Signal for the association between HCY and ED

Although after comprehensive analyses, no significant association between HCY levels and ED was discovered (**Table 2-5**). When grouping the participants according to age, we found that HCY might be associated with ED, especially in the old men (age ≥ 60) (**Table S2**). Similar relevance was confirmed in the marital status (alone, Unadjusted severe ED: OR = 4.385, 95% CI = 1.070-17.974, $P = 0.040$; Age-adjusted severe ED: OR = 5.085, 95% CI = 1.195-21.636, $P = 0.028$) (**Table S3**).

Then, the HCY was divided into normal (HCY 5-15 μmol/L) and hyperhomocysteinemia (HCY > 15 μmol/L). Similarly, the significant association between HCY and ED seemed to be more prominent in the men living alone (Age-adjusted severe ED: OR = 2.448, 95% CI = 1.046-5.733, $P = 0.039$) (**Table S3**).

B12 level significantly associated with ED

In the process of investigating the association between ED and B12, linear and logistic regression analyses were also applied. No significant results were detected for B12 in linear regression analysis (in which IIEF-5 scores were treated as the dependent factor). As for the binary logistic regression (the status of ED evaluated by IIEF-5 was treated as the dependent

factor), B12 was identified to be associated with ED in the Unadjusted model (OR = 1.438, 95% CI = 1.070-1.933, P = 0.016). However, the association disappeared in other adjusted models (**Table 3**). Furthermore, we tried to discover the relationship between B12 and ED, based on the severity grades of ED. Interestingly, the positive correlation between B12 and ED was confirmed again, especially among men 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**). Then, the level of B12 was divided into quartiles. The result 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; and P for trend < 0.001) (**Table 5**).

As shown in the analyses above, after adjusting for age, the significant association between B12 and ED disappeared (**Table 2 and 3** and **Table 5**), which suggested that age cannot be ignored in investigating the relationship between B12 and ED. 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 correlations between B12 and mild ED (IIEF-5 = 17–21) mainly presented in the 40–49 years old age group (OR = 2.907, 95% CI = 1.402-6.026, P = 0.004) (**Table S2**).

Our baseline analysis discovered different proportions of educational status in the ED and Non-ED groups. In order to discuss the influences of marital and educational status in relation to ED and B12, we performed further between-group analyses. Similar to previous results, B12 was also identified to be associated with mild ED, even after multivariate adjustment (marital status, living together: OR = 1.501, 95% CI = 1.035-2.175, P = 0.032; alone: OR = 3.449, 95% CI = 1.113-10.692, P = 0.032; and educational status, Intermediate: OR = 1.858, 95% CI = 1.214-2.845, P = 0.004) (**Table S3**).

Discussion

ED is a common disease, affecting a large number of males. [1–4] Recent studies suggest HCY may be an independent risk factor for ED. [8, 9] In order to test this association, the study conducted is based on the larger population-based FAMHES project. After analysis, HCY is confirmed to be associated with ED, especially severe ED. Moreover, B12 may also be related to mild ED. However, no significant association between FA and ED is discovered in our study.

HCY is said to be associated with many diseases and health conditions, such as psychological disorders, [17, 18] lipid profiles, [19] renal Impairment, [20] and inflammatory/immune factors. [21] Additionally, HCY is also identified to be a useful marker for CVD. [22, 23] Meanwhile, ED could be a potentially predictive factor for cardiovascular and other chronic diseases. [24] Based on this relevance, it is said that HCY might be a risk factor for ED. [8, 9] As expected, we identified that HCY was significantly associated with ED, especially severe ED. The main mechanism might be that HCY could influence endothelial dysfunction and nitric oxide (NO) diffusion. Previously, in vitro and in vivo studies discovered that HCY could be a toxin for the vasculature by inducing endothelial dysfunction. [25] Additionally, NO mainly participates in vascular dilation, smooth muscle relaxation (including genitourinary smooth muscle), and permitting penile erection. [26, 27] Studies showed that increased HCY could inhibit NO synthase (which could influence the production of NO), influencing the development of ED. [28] So, on the basis of these processes, we could understand the risk effect of HCY in inducing ED. Additionally, the status of living alone

for men would also influence this association, which hinted the pathogenesis of psychological factors for ED.

B12 is also known as cobalamin. Similar to FA, it is an important cofactor in methionine synthesis and homocysteine metabolism. [29] Although previous studies had identified that FA might be a potential protective factor for ED, [30] no significant association has been detected this time. As for B12, opposite to HCY, it has been said to protect against ED. [31] Although, our study also 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 possible 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, defense mechanisms are triggered. As a potential protective factor, the absorption of B12 is enhanced. Combining the 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.

Limitations

Our study verified the previous conclusions that HCY could increase the risk of ED. However, some limitations still need to be noted: (i) this study is a cross-sectional analysis, which just reflects the status of specific time points and populations; (ii) there are limited numbers of participants with primary educational status. So, the results are exaggerated, which needs to be examined further; (iii) although we have identified a significant association between B12 and ED, the exact mechanisms and effects were unclear until now; and (iv) after multiple testing, no positive association can be detected, which suggests that our results might be unstable. So, further studies will be unstable.

Conclusions

ED is one of the most common male diseases. This study was conducted in order to discover the functions of HCY, B12, and FA in ED. Our results confirmed the pathogenic effect of HCY on ED, especially for severe ED. Meanwhile, B12 might also be significantly associated with ED. Further studies with larger pools of participants should be focused on the potential mechanisms and therapeutic effects of B12 on ED.

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Conflict of Interest

There are no conflicts of interests.

Author Contributions

Y.C., J.L., T.Y.L., Z.N.M., and J.W.C. participated in participants' collection, field investigation, design, writing and modification of all the paper. Y.C. and J.L. took part in the statistical analysis. Z.N.M. and J.W.C. provided important advices for this paper. J.X.L., and J.L.L. provide efforts in the processes of modification.

Data Sharing Statement

The data for this study was available in the supplementary materials. Further questions could be sent to ZN.M (zengnanmo@hotmail.com) and JW.C (chengjiwen1977@foxmail.com).

References

1. Braun M, Wassmer G, Klotz T, et al. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res 2000;12:305-11. [PubMed: 11416833]
2. Pinnock CB, Stapleton AM, Marshall VR. Erectile dysfunction in the community: a prevalence study. Med J Aust 1999;171:353-7. [PubMed: 10590723]
3. Nicolosi A, Moreira ED Jr, Shirai M, et al. Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectile dysfunction. Urology 2003;61:201-6. [PubMed: 10590723]
4. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 1999;84:50-56. [PubMed: 10444124]
5. Kloner RA, Speakman M. Erectile dysfunction and atherosclerosis. Curr Atheroscler Rep 2002;4:397-401. [PubMed: 12162940]
6. Sullivan ME, Keoghane SR, Miller MA. Vascular risk factors and erectile dysfunction. BJU Int 2001;87:838-45. [PubMed: 11412223]
7. Nehra A, Jackson G, Miner M, et al. Diagnosis and treatment of erectile dysfunction for reduction of cardiovascular risk. J Urol 2013;189:2031-8. [PubMed: 23313195]
8. 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. [PubMed: 10510122]
9. 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. [PubMed: 27709655]
10. Long Y, Nie J. Homocysteine in Renal Injury. Kidney Dis (Basel) 2016; 2:80-7. [PubMed: 27536696]
11. Lai WK, Kan MY. Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1-12. [PubMed: 26201664]
12. Sansone M, Sansone A, Romano M, et al. Folate: a possible role in erectile dysfunction? Aging Male. 2017 Nov 20:1-5. [PubMed: 29157083]
13. 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. [PubMed: 25343742]
14. Rosen RC, Cappelleri JC, Smith MD, et al. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for

erectile dysfunction. Int J Impot Res 1999;11:319-26. [PubMed: 10637462]
15. Kupelian V, Araujo AB, Chiu GR, et al. Relative contributions of modifiable risk factors to erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. Prev Med 2010;50:19-25. [PubMed: 19944117]
16. Guo H, Chi J, Xing Y, et al. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res 2009; 129:279-84. [PubMed: 19491420]
17. Salagre E, Vizuete AF, Leite M, et al. Homocysteine as a peripheral biomarker in bipolar disorder: A meta-analysis. Eur Psychiatry. 2017;43:81-91. [PubMed: 28371745]
18. Elstgeest LE, Brouwer IA, Penninx BW, et al. Vitamin B12, homocysteine and depressive symptoms: a longitudinal study among older adults. Eur J Clin Nutr 2017;71:468-75. [PubMed: 28145420]
19. Momin M, Jia J, Fan F, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis 2017; 16:54. [PubMed: 28288621]
20. Chen J, Li G, Xu Z, et al. Elevated Plasma Homocysteine Level Increased the Risk of Early Renal Impairment in Acute Ischemic Stroke Patients. Cell Mol Neurobiol 2017;37:1399-1405. [PubMed: 28275883]
21. Li T, Chen Y, Li J, et al. Serum Homocysteine Concentration Is Significantly Associated with Inflammatory/Immune Factors. PLoS One 2015; 10:e0138099. [PubMed: 26367537]
22. Sahu A, Gupta T, Kavishwar A, et al. Cardiovascular Diseases Risk Prediction by Homocysteine in Comparison to other Markers: A Study from Madhya Pradesh. J Assoc Physicians India 2015;63:37-40. [PubMed: 27608690]
23. Yeh JK, Chen CC, Hsieh MJ, et al. Impact of Homocysteine Level on Long-term Cardiovascular Outcomes in Patients after Coronary Artery Stenting. J Atheroscler Thromb 2017;24:696-705. [PubMed: 27803490]
24. Baumann F, Hehli D, Makaloski V, et al. Erectile dysfunction - overview from a cardiovascular perspective. Vasa 2017; 1-7. [PubMed: 28486869]
25. McDowell IF, Lang D. Homocysteine and endothelial dysfuncton: A link with cardiovascular disease. J Nutr 2000;130: 369S-372S. [PubMed: 10721909]
26. Rajfer J, Aronson WJ, Bush PA, et al. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Engl J Med 1992;326:90-94. [PubMed: 1309211]
27. Deanfield J, Donald A, Ferri C, et al. Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension: Endothelial function and dysfunction. Part I: methodological issues for assessment in the different vascular beds: a statement by the working group on endothelin and endothelial factors of the European society of hypertension. J Hypertens 2005;23:7-17. [PubMed: 15643116]
28. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-375. [PubMed: 10475890]
29. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2:299-316. [PubMed: 22254022]
30. Yan WJ, Yu N, Yin TL, et al. A new potential risk factor in patients with erectile dysfunction

and premature ejaculation: folate deficiency. Asian J Androl 2014;16:902-906. [PubMed: 25080932]

31. Giovannone R, Busetto GM, Antonini G, et al. Hyperhomocysteinemia as an Early Predictor of Erectile Dysfunction: International Index of Erectile Function (IIEF) and Penile Doppler Ultrasound Correlation With Plasma Levels of Homocysteine. Medicine (Baltimore) 2015;94:e1556. [PubMed: 26426624]

	ED	Non-ED	P
No.	688	693	
Age, years	**37.99±10.75**	**34.18±8.47**	**<0.001[b]**
BMI, Kg/m^2	23.27±3.26	23.37±3.48	0.591[b]
WHR	0.88±0.06	0.88±0.06	0.253[b]
HCY, μmol/L	14.97±4.11	15.34±11.09	0.524[b]
Normal HCY	392 (56.98%)	433 (62.48%)	
Hyperhomocysteinemia	**296 (43.02%)**	**260 (37.52%)**	**0.037[b]**
B12, pg/ml	**718.53±234.37**	**688.74±229.68**	**0.015[b]**
FA, ng/ml	9.56±2.72	9.89±11.28	0.594[b]
Smoke			
Yes	392 (56.98%)	385 (55.56%)	
No	296 (43.02%)	308 (44.44%)	0.594[c]
Drink			
Yes	**586 (85.17%)**	**617 (89.03%)**	
No	**102 (14.83%)**	**76 (10.97%)**	**0.032[c]**
Marital status[e]			
Live together	595 (86.48%)	578 (83.41%)	
Alone	93 (13.52%)	115 (16.59%)	0.110[c]
educational status[a]			
Primary	**19 (2.76%)**	**5 (0.72%)**	
Intermediate	**488 (70.93%)**	**411 (59.39%)**	
Superior	**181 (26.31%)**	**276 (39.89%)**	**<0.001[c]**

Table 1. The characteristics of the eligible participants in the analysis

a. One participant without the information of educational status in the Non-ED group

b. Student's t-test

c. chi-square test

e. The marital status was classified into live together (married or cohabitation without marriage) and alone (spinsterhood or widowed).

* Normal HCY: 5-15μmol/L; hyperhomocysteinemia: >15umol/L

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

	Unadjusted			Age-adjusted			Multivariate adjusted		
	BETA	95%CI	P	BETA	95%CI	P	BETA	95%CI	P
IIEF-5									
HCY	-0.202	-1.080, 0.676	0.651	0.139	-0.732, 1.009	0.755	0.084	-0.787, 0.956	0.850
Binary HCY	-0.338	-0.817, 0.142	0.167	-0.146	-0.622, 0.330	0.548	-0.186	-0.663, 0.291	0.444
B12	0.048	-0.600, 0.696	0.885	0.212	-0.428, 0.852	0.515	0.404	-0.259, 1.068	0.232
FA	0.112	-0.668, 0.891	0.779	0.388	-0.384, 1.160	0.986	0.324	-0.496, 1.145	0.438

Table 2. The linear regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* IIEF-5 scores were the dependent factor for the linear regression analysis.

	Unadjusted			Age-adjusted			Multivariate adjusted		
Binary	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY	1.137	0.766-1.687	0.524	0.959	0.641-1.435	0.839	0.986	0.658-1.479	0.986
Binary HCY	**1.258**	**1.014-1.560**	**0.037**	1.151	0.923-1.435	0.212	1.174	0.940-1.466	0.157
B12	**1.438**	**1.070-1.933**	**0.016**	1.338	0.992-1.805	0.057	1.311	0.961-1.788	0.087
FA	1.100	0.775-1.561	0.594	0.956	0.668-1.367	0.804	1.041	0.710-1.527	0.835

Table 3. The binary regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* In the binary regression analysis, the ED status (ED: IIEF-5≤21; Non-ED: IIEF-5>21) was treated as the dependent factor.

	HCY			Binary_HCY			B12			FA		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
ED-Unadjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	1.081	0.695-1.681	0.730	1.246	0.980-1.583	0.072	**1.694**	**1.207-2.376**	**0.002**	1.180	0.801-1.740	0.402
Moderate (12–16)	1.258	0.649-2.439	0.497	1.298	0.896-1.880	0.168	1.187	0.715-1.972	0.508	0.960	0.519-1.776	0.896
Severe (5–11)	1.259	0.562-2.820	0.575	1.258	0.799-1.980	0.322	0.891	0.496-1.599	0.698	0.923	0.434-1.963	0.834
ED-age-adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.948	0.607-1.480	0.814	1.166	0.914-1.486	0.217	**1.596**	**1.135-2.244**	**0.007**	1.052	0.710-1.558	0.800
Moderate (12–16)	0.929	0.455-1.898	0.840	1.095	0.747-1.605	0.643	1.409	0.635-1.733	0.851	0.762	0.404-1.437	0.401
Severe (5–11)	1.068	0.467-2.443	0.876	1.150	0.726-1.820	0.552	0.839	0.471-1.495	0.551	0.794	0.370-1.705	0.554
ED- Multivariate adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.973	0.621-1.524	0.904	1.188	0.929-1.517	0.169	**1.620**	**1.141-2.300**	**0.007**	1.184	0.775-1.808	0.435
Moderate (12–16)	0.965	0.472-1.971	0.922	1.119	0.762-1.643	0.567	0.972	0.576-1.640	0.915	0.777	0.401-1.507	0.456
Severe (5–11)	1.132	0.491-2.613	0.771	1.187	0.748-1.885	0.467	0.717	0.388-1.324	0.288	0.821	0.368-1.831	0.631

Table 4. Multinomial logistic regression for the association between ED and HCY, B12 and FA

* The symptoms of ED were divided into None (IIEF-5= 22-25), Mild (17-21), Moderate (12-16) and Severe (5-11). And the None (22-25) was the reference.

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

	Unadjusted			Age-adjusted			Multivariate adjusted		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.130	0.838-1.525	0.422	1.022	0.753-1.387	0.887	1.012	0.744-1.377	0.938
Q3	1.292	0.958-1.743	0.094	1.187	0.875-1.610	0.271	1.210	0.890-1.646	0.224
Q4	1.276	0.946-1.720	0.110	1.091	0.803-1.483	0.578	1.103	0.810-1.502	0.534
B12									
Q1	1	1	1	1	1	1	1	1	1
Q2	0.917	0.680-1.236	0.569	0.893	0.659-1.209	0.464	0.899	0.662-1.221	0.496
Q3	0.988	0.733-1.333	0.939	0.972	0.717-1.316	0.853	0.986	0.726-1.338	0.927
Q4	**1.452**	**1.076-1.961**	**0.015**	1.299	0.955-1.765	0.095	1.286	0.945-1.752	0.110
FA									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.313	0.974-1.770	0.074	1.325	0.978-1.795	0.069	1.332	0.981-1.811	0.067
Q3	1.300	0.963-1.755	0.086	1.243	0.916-1.687	0.163	1.286	0.944-1.751	0.111
Q4	1.198	0.888-1.616	0.236	1.094	0.806-1.487	0.564	1.116	0.819-1.522	0.487

Table 5. Association between HCY, B12, FA and ED along with the increased levels of these indexes

* Multinomial logistic regression was applied.

* The levels of HCY, B12, and FA was divided into quartile (Q1<levels of 25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%).

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

Excluded diseases	Excluded drugs
Hypertension	Vitamins: Vitamins B12, Vitamins C, Vitamins B2, etc.
Diabetes	Antidiabetic medicines: insulin, metformin, Ningestedglinide, etc.
Angina	Non-steroidal anti-inflammatory drugs: aspirin, acetaminophen, indomethacin, diclofenac, celecoxib, etc.
Myocardial infarction	Antibiotics: penicillin, cephalosporins, aztreonam, ofloxacin, clarithromycin, etc.
Stroke	Cimetidine:
Gout	Glucocorticoids: hydrocortisone, prednisone, methylprednisolone, hexadecadrol, etc.
Rheumatoid arthritis	
Prostatitis	
Hepatitis B	
Various cancers	
Parotiditis	
Urolithiasis	
Pelvic floor surgery	

Table S1. The list of diseases and drugs excluded in this study

		No. ED	No. Non-ED	HCY			Binary HCY			B12			FA		
				BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P
Ages <40															
IIEF-5		409	531	-0.246	-1.32, 0.837	0.656	-0.269	-0.845, 0.307	0.359	0.627	-0.202, 1.456	0.138	-0.137	-1.135, 0.862	0.788
ED				0.987	0.594-1.639	0.960	1.147	0.876-1.501	0.319	1.207	0.818-1.780	0.344	1.146	0.718-1.829	0.567
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.961	0.547-1.688	0.889	1.185	0.881-1.594	0.263	1.460	0.945-2.255	0.088	1.162	0.692-1.950	0.571
	Moderate			0.794	0.282-2.238	0.662	0.843	0.486-1.462	0.544	0.957	0.444-2.066	0.912	1.015	0.401-2.568	0.974
	Severe			1.429	0.469-4.357	0.530	1.351	0.732-2.495	0.336	0.544	0.223-1.326	0.180	1.276	0.432-3.766	0.659
40-49		176	131												
IIEF-5				0.501	-1.025, 2.207	0.519	-0.109	-1.060, 0.841	0.821	0.320	-0.904, 1.544	0.607	0.990	-0.622, 2.602	0.228
ED				0.842	0.398,-1.778	0.651	1.129	0.710-1.795	0.608	1.692	0.925-3.094	0.088	0.878	0.398-1.937	0.747
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.820	0.358-1.878	0.638	1.135	0.685-1.882	0.623	**2.907**	**1.402-6.026**	**0.004**	1.174	0.495-2.785	0.716
	Moderate			0.951	0.288-3.146	0.935	1.267	0.598-2.682	0.537	0.550	0.224-1.355	0.194	0.380	0.102-1.407	0.147
	Severe			0.845	0.140-5.123	0.855	0.905	0.318-2.578	0.852	0.910	0.243-3.403	0.889	0.579	0.105-3.183	0.530
50-59		69	21												
IIEF-5				4.297	-0.514, 9.108	0.079	1.597	-0.408, 3.602	0.117	-2.046	-4.862, 0.770	0.152	-1.649	-5.387, 2.088	0.383
ED				0.314	0.028-3.584	0.351	0.867	0.309-2.430	0.786	1.521	0.355-6.516	0.572	1.773	0.265-11.867	0.555
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.379	0.024-5.901	0.489	0.927	0.299-2.875	0.895	0.859	0.171-4.308	0.853	1.601	0.198-12.945	0.659
	Moderate			0.669	0.030-14.774	0.799	1.326	0.356-4.931	0.674	4.335	0.603-31.187	0.145	1.042	0.086-12.647	0.974
	Severe			0.046	0.001-1.941	0.107	0.358	0.077-1.660	0.189	2.060	0.256-16.573	0.497	3.751	0.263-53.516	0.330
≥60		34	10												
IIEF-5				-0.479	-7.400, 6.442	0.889	-0.116	-3.491, 3.259	0.945	-0.526	-4.308, 3.255	0.779	2.899	-2.726, 8.523	0.303
ED				29.170	0.379-2.243E3	0.128	2.544	0.409-15.822	0.317	0.714	0.089-5.723	0.751	0.523	0.018-15.079	0.705
	None			1	1	1	1	1	1	1	1	1	1	1	1

	Mild	**767.519**	**1.649-3.573E5**	**0.034**	4.093	0.337-49.760	0.269	0.677	0.038-12.139	0.791	0.645	0.008-52.675	0.845
	Moderate	11.236	0.098-1.289E3	0.317	2.266	0.283-18.119	0.441	1.067	0.092-12.408	0.959	2.717	0.049-150.689	0.626
	Severe	70.920	0.291-1.729E4	0.129	3.281	0.316-34.092	0.320	0.424	0.037-4.866	0.491	0.027	0.000-3.577	0.148

Table S2. Association between ED and HCY, B12, FA level in four ages groups (<40, 40-49, 50-59 and ≥60 years) after adjusting multivariate

* Linear regression for the IIEF-5; binary regression analysis for ED; multinomial logistic regression for the severity of symptom of ED

* ED is divided into four groups according to the IIEF-5 scores: None=22-25 scores, Mild=17-21 scores, Moderate=12-16 scores and Severe=5-11 scores. None ED is treated as the reference.

* ED: erectile dysfunction; HCY= homocysteine; B12= vitamin B12; FA= folic acid; BMI: body mass index; WHR: waist hip rate; OR: odd ratio; 95%CI: 95% confidence interval

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* Multi-adjusted: age, BMI, WHR, smoke and drink

	ED grading	HCY			Binary_HCY			B12			FA		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Unadjusted													
Marital status													
Live together		1.070	0.698-1.640	0.757	1.237	0.979-1.562	0.074	**1.400**	**1.010-1.940**	**0.044**	1.283	0.860-1.916	0.222
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.077	0.673-1.722	0.758	1.239	0.959-1.601	0.101	**1.484**	**1.033-2.132**	**0.033**	1.295	0.833-2.012	0.251
	Moderate	1.267	0.629-2.555	0.508	1.367	0.920-2.032	0.122	1.105	0.636-1.921	0.723	1.158	0.583-2.303	0.675
	Severe	0.678	0.227-2.027	0.487	0.987	0.566-1.722	0.964	1.534	0.697-3.375	0.288	1.492	0.580-3.841	0.407
Alone		1.523	0.540-4.299	0.426	1.346	0.765-2.370	0.303	1.458	0.705-3.013	0.309	0.577	0.246-1.356	0.207
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.813	0.207-3.194	0.767	1.169	0.580-2.355	0.662	**3.950**	**1.331-11.719**	**0.013**	0.750	0.285-1.976	0.561
	Moderate	0.985	0.128-7.560	0.988	0.812	0.267-2.470	0.714	1.568	0.352-6.975	0.555	0.330	0.058-1.878	0.211
	Severe	**4.385**	**1.070-17.974**	**0.040**	2.249	0.974-5.193	0.058	0.605	0.252-1.450	0.260	0.460	0.116-1.824	0.269
educational status													
Primary		1.586	0.018-142.652	0.841	0.875	0.116-6.579	0.897	5.169	0.188-142.118	0.331	0.399	0.095-1.676	0.209
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	3.257	0.018-596.353	0.657	0.500	0.045-5.514	0.571	4.007	0.136-117.975	0.421	0.455	0.088-2.364	0.349
	Moderate	0.489	0.002-114.662	0.797	1.500	0.136-16.542	0.741	**1.266E^{3}**	**2.303-6.962E^{5}**	**0.026**	0.423	0.060-2.975	0.387
	Severe	2.199	0.007-686.953	0.788	1.000	0.080-12.557	1.000	2.627	0.200-34.591	0.463	0.205	0.009-4.472	0.314
Intermediate		1.667	0.989-2.811	0.055	1.305	0.993-1.716	0.056	**1.513**	**1.049-2.182**	**0.027**	1.187	0.747-1.887	0.469
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.571	0.883-2.795	0.124	1.295	0.958-1.752	0.093	**1.838**	**1.215-2.781**	**0.004**	1.304	0.779-2.181	0.312
	Moderate	1.619	0.692-3.785	0.266	1.265	0.806-1.986	0.307	1.132	0.620-2.067	0.687	0.888	0.411-1.919	0.763
	Severe	2.355	0.876-6.326	0.089	1.429	0.829-2.463	0.199	0.934	0.452-1.926	0.852	1.160	0.451-2.980	0.759
Superior		0.874	0.449-1.699	0.691	1.436	0.986-2.093	0.060	0.938	0.537-1.640	0.823	1.117	0.586-2.129	0.738
	None	1	1	1	1	1	1	1	1	1	1	1	1

	Mild	0.765	0.351-1.669	0.501	1.403	0.924-2.130	0.112	1.156	0.612-2.183	0.655	1.202	0.587-2.462	0.615
	Moderate	1.581	0.567-4.414	0.381	1.800	0.849-3.818	0.126	0.640	0.225-1.821	0.403	1.244	0.345-4.481	0.739
	Severe	0.479	0.064-3.567	0.472	1.170	0.461-2.970	0.741	0.491	0.142-1.691	0.259	0.569	0.116-2.788	0.487
Age-Adjusted													
Marital status													
Live together		9.868	0.559-1.347	0.528	1.110	0.873-1.412	0.394	1.333	0.954-1.861	0.092	1.122	0.743-1.694	0.584
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.940	0.586-1.508	0.796	1.157	0.892-1.501	0.271	1.436	0.996-2.072	0.053	1.176	0.752-1.839	0.478
	Moderate	0.874	0.402-1.902	0.735	1.110	0.735-1.676	0.620	1.015	0.583-1.766	0.958	0.947	0.469-1.909	0.878
	Severe	0.378	0.114-1.258	0.113	0.762	0.428-1.357	0.356	1.353	0.622-2.943	0.445	1.193	0.459-3.097	0.717
Alone		1.611	0.566-4.586	0.371	1.406	0.793-2.493	0.243	1.391	0.653-2.961	0.392	0.622	0.257-1.506	0.292
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.216-3.352	0.817	1.210	0.598-2.451	0.596	**3.742**	**1.258-11.129**	**0.018**	0.799	0.294-2.169	0.659
	Moderate	1.019	0.132-7.836	0.986	0.831	0.272-2.542	0.745	1.492	0.339-6.562	0.597	0.341	0.060-1.947	0.226
	Severe	**5.085**	**1.195-21.636**	**0.028**	**2.448**	**1.046-5.733**	**0.039**	0.450	0.175-1.159	0.098	0.516	0.122-2.174	0.367
educational status													
Primary		0.285	0.001-61.236	0.647	0.212	0.010-4.431	0.317	10.044	0.027-3.674E^{3}	0.444	0.421	0.080-2.228	0.309
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.588	0.004-601.127	0.879	0.228	0.010-5.326	0.357	4.021	0.062-259.697	0.513	0.485	0.093-2.542	0.392
	Moderate	0.001	7.773E^{-8}-6.096	0.116	0.139	0.003-6.581	0.316	3.874E^{3}	1.164-1.289E^{7}	0.046	0.426	0.024-7.587	0.561
	Severe	0.035	1.430E^{-5}-84.089	0.398	0.118	0.002-6.145	0.290	8.466	0.008-8.474E^{3}	0.545	0.162	0.005-5.514	0.311
Intermediate		1.392	0.820-2.365	0.221	1.209	0.915-1.597	0.182	1.384	0.954-2.009	0.087	1.073	0.669-1.722	0.769
	None	1	1	1	1	1		1	1	1	1	1	1
	Mild	1.382	0.774-2.468	0.275	1.229	0.906-1.668	0.186	**1.723**	**1.133-2.620**	**0.011**	1.206	0.717-2.030	0.480
	Moderate	1.145	0.470-2.787	0.766	1.084	0.682-1.724	0.733	0.966	0.529-1.764	0.911	0.754	0.346-1.643	0.478
	Severe	1.915	0.698-5.257	0.207	1.298	0.748-2.254	0.354	0.840	0.409-1.726	0.635	1.022	0.397-2.632	0.964
Superior		0.779	0.391-1.552	0.477	1.324	0.900-1.948	0.154	0.953	0.540-1.684	0.869	0.898	0.461-1.750	0.751

	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.672	0.297-1.521	0.340	1.289	0.841-1.976	0.245	1.175	0.618-2.238	0.623	0.960	0.458-2.012	0.914
	Moderate	1.421	0.486-4.155	0.521	1.583	0.736-3.405	0.240	0.682	0.241-1.926	0.470	0.917	0.244-3.440	0.898
	Severe	0.521	0.074-3.647	0.511	1.202	0.472-3.061	0.699	0.473	0.132-1.690	0.249	0.622	0.125-3.096	0.562
Multivariate adjusted													
Marital status													
Live together		0.894	0.575-1.390	0.619	1.137	0.893-1.448	0.299	1.349	0.962-1.891	0.083	1.176	0.773-1.789	0.449
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.963	0.598-1.548	0.875	1.181	0.909-1.534	0.213	**1.501**	**1.035-2.175**	**0.032**	1.245	0.790-1.961	0.345
	Moderate	0.913	0.420-1.985	0.818	1.136	0.751-1.719	0.547	0.971	0.554-1.701	0.918	0.933	0.455-1.915	0.851
	Severe	0.417	0.126-1.378	0.152	0.812	0.454-1.453	0.483	1.227	0.562-2.675	0.608	1.317	0.494-3.514	0.582
Alone		1.602	0.546-4.698	0.390	1.399	0.779-2.512	0.261	1.288	0.558-2.975	0.553	0.678	0.253-1.820	0.441
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.206-3.498	0.821	1.199	0.579-2.481	0.625	**3.449**	**1.113-10.692**	**0.032**	0.959	0.279-3.292	0.947
	Moderate	1.130	0.135-9.481	0.910	0.856	0.276-2.654	0.788	1.244	0.261-5.929	0.784	0.336	0.054-2.069	0.239
	Severe	4.181	0.951-19.374	0.058	2.356	0.989-5.616	0.053	0.302	0.084-1.083	0.066	0.598	0.139-2.570	0.490
educational status													
Primary		0.045	0.000-51.900	0.388	0.098	0.002-5.651	0.262	6.248	0.006-6.418E3	0.605	2.823	0.032-247.907	0.649
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.198	$4.915E^{-5}$-794.766	0.702	0.224	0.004-13.29	0.473	0.001	3.247E-10-3.819E3	0.376	1.806	0.007-479.698	0.836
	Moderate	$2.184E^{-12}$	$2.988E^{-28}$-$1.597E^{4}$	0.150	0.004	1.429E-6-8.838	0.157	**NA**	**NA**	**0.043**	3.396	$0.006\text{-}1.820E^{3}$	0.703
	Severe	1.972E-9	$1.318E^{-24}$-$2.952E^{6}$	0.261	0.008	$4.142E^{-6}$-15.343	0.210	**$8.065E^{-217}$**	**0.000-0.203**	**0.049**	0.516	0.001-457.324	0.849
Intermediate		1.431	0.842-2.432	0.186	1.223	0.925-1.618	0.158	1.428	0.981-2.081	0.063	1.092	0.677-1.764	0.717
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.401	0.782-2.510	0.257	1.238	0.910-1.682	0.174	**1.858**	**1.214-2.845**	**0.004**	1.268	0.748-2.149	0.378
	Moderate	1.225	0.502-2.990	0.656	1.105	0.694-1.761	0.673	0.954	0.519-1.755	0.880	0.707	0.319-1.568	0.394
	Severe	2.044	0.744-5.615	0.165	1.350	0.776-2.351	0.288	0.754	0.364-1.562	0.448	0.969	0.367-2.562	0.950

Superior		0.812	0.405-1.627	0.557	1.360	0.920-2.011	0.123	0.977	0.550-1.738	0.938	0.889	0.449-1.762	0.737
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.702	0.308-1.598	0.399	1.325	0.859-2.044	0.204	1.202	0.632-2.287	0.575	0.898	0.419-1.926	0.783
	Moderate	1.437	0.483-4.270	0.514	1.556	0.718-3.370	0.262	0.647	0.217-1.930	0.435	1.056	0.274-4.063	0.937
	Severe	0.598	0.092-3.890	0.591	1.333	0.513-3.459	0.555	0.496	0.123-2.002	0.325	0.715	0.145-3.529	0.681

Table S3. The logistic regression analyses (binary and multinomial) for the association between ED and HCY, B12 and FA in the subgroups of marital status and educational status

* In the educational status, the Primary group only contains 24 participants. So, some results of regression analyses were exaggerated with these limited data

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15µmol/L); hyperhomocysteinemia (>15umol/L)

* ED status: none (IIEF-5 score 22–25); mild (17–21); moderate (12–16); and severe (5–11).

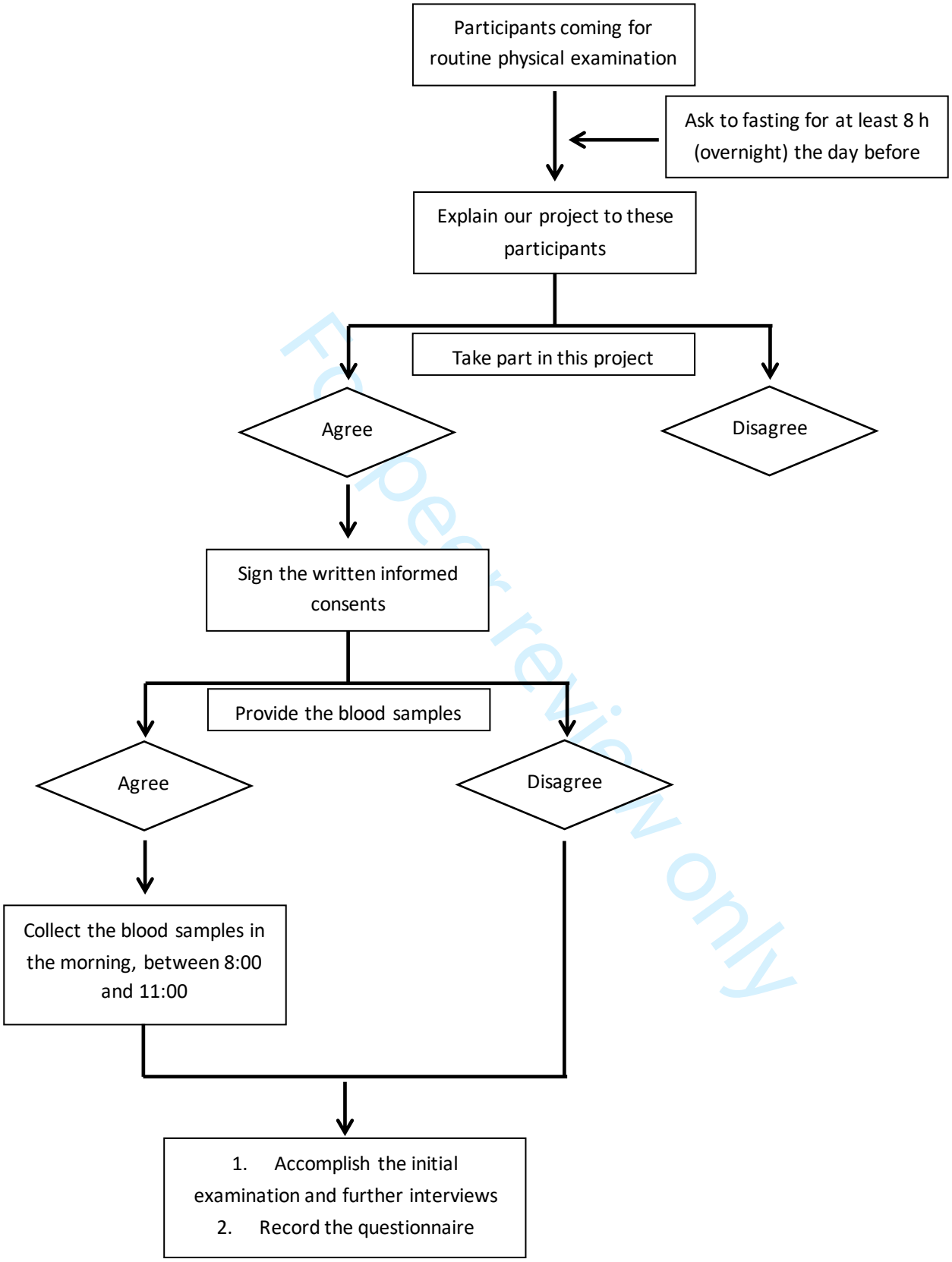

Figure S1. The flow of participants' collection

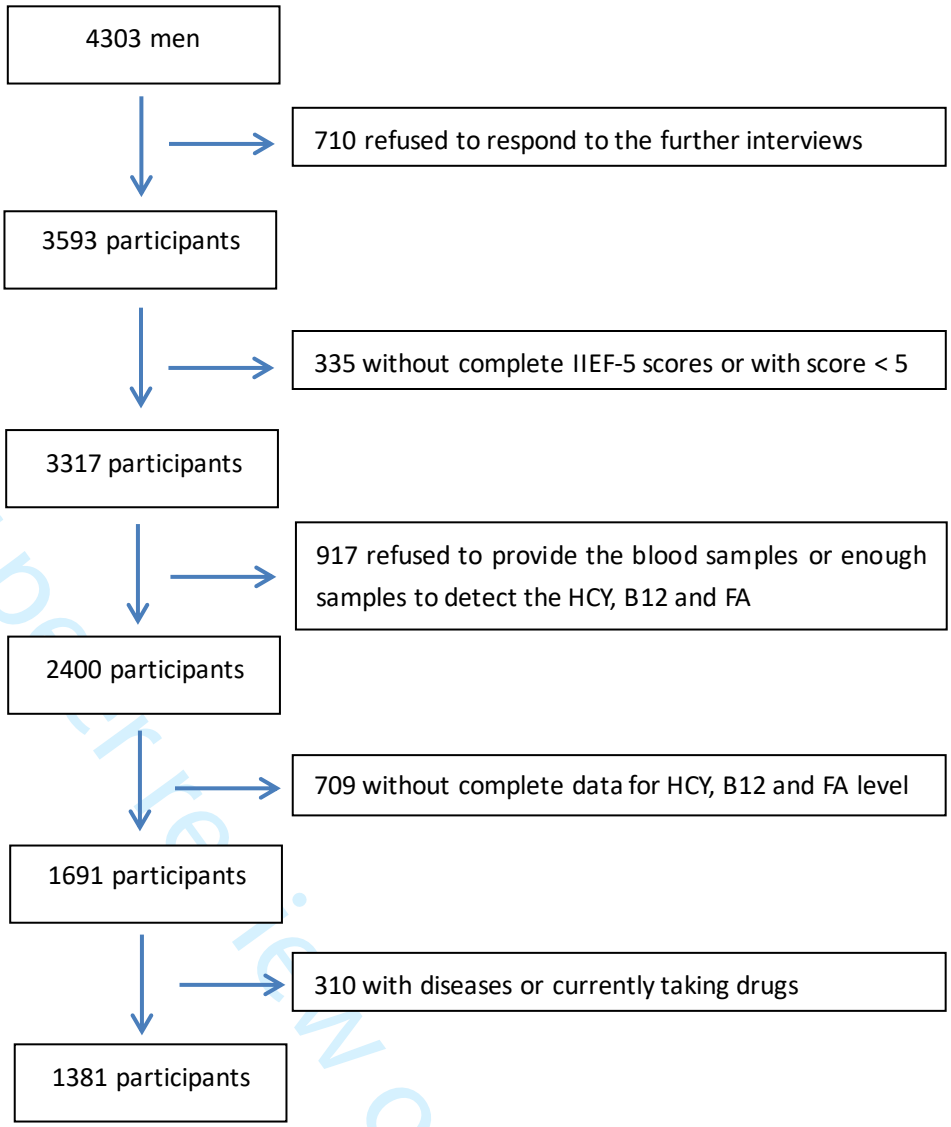

Figure S2. The flow for screening the eligible participants

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	*4*
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6
		(*b*) Report category boundaries when continuous variables were categorized	5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	7

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study in China

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study in China

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Abstract

Objectives: Erectile dysfunction (ED) affects up to 53.4% of men aged 30-80 years. In this study, we aimed to examine the association between homocysteine (HCY), vitamin B12 (B12), folic acid (FA), and ED.

Design: Cross-sectional study.

Setting: Guangxi, China.

Participants: A total of 1381 participants completed questionnaires for the International Index of Erectile Function (IIEF-5) scores, and the values of HCY, B12 and FA between September 2009 and December 2009.

Measures: ED was evaluated by IIEF-5. Regression and between-group analyses were performed.

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 (bachelors or bachelorhood). 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.

Keywords: erectile dysfunction, homocysteine, vitamin B12, folic acid

Strengths and limitations of this study

1. Our study is a cross-sectional study, mainly based on the large Fangchenggang Area Male Health and Examination Survey (FAMHES) project, including a total of 4303 men.
2. This study includes comprehensive analyses of baseline, linear and logistic regression, and multinomial logistic regression.
3. According to the changes in the HCY, B12, and FA levels, and the order of ED severity, we investigated the associations between HCY, B12, FA, and ED.
4. The study also took into consideration of the effects of age, marital and educational status.
5. Nevertheless, as a cross-sectional analysis, the exact mechanisms of the relationship between HCY, B12, FA, and ED cannot be clearly defined.

Introduction

Erectile dysfunction (ED) is defined as the inability to acquire and maintain satisfying sexual intercourse with a sufficient erection, affecting up to 53.4% of men aged 30 to 80 years. [1] The morbidity increases sharply among men over 40 years of age. [2, 3] It has been estimated that the prevalence of ED will reach 322 million worldwide by the year 2025. [4]

Various factors including smoking, hypertension, and hyperlipidemia have been identified to influence the development of ED. Among these factors, the vascular component is dominant. [5, 6] Moreover, ED may be one of the indicators of cardiovascular disease (CVD). [7] Homocysteine (HCY) , a CVD-associated factor was recently defined as an independent risk factor for ED. [8, 9] HCY is a thiol-containing amino acid, mainly from methionine, with two steps of transformation. First, methionine is catalyzed to form S-adenosylmethionine (SAM) by the enzyme SAM synthase. As a major methyl group donor for various methylation reactions, SAM is mainly transformed into S-adenosylhomocysteine (SAH) after loss of the methyl group. In the second step, SAH is hydrolyzed by SAH hydrolase to form HCY and adenosine. Biologically, HCY is involved in two pathways, including remethylation (RM) and transsulfuration (TS). In the RM pathway, HCY regenerates methionine by methylenetetrahydrofolate reductase (MTHFR) with cofactors of folic acid (FA) and vitamin B12 (B12). In the TS pathway, HCY is catalyzed by the cystathione-β-synthase (CBS) and γ-cystathionase. [10, 11]

FA and B12 as the cofactors of HCY, have also been identified to be associated with ED. [12] However, limited studies have been focused on the relevance of their levels to ED. On the basis of previous studies, we hypothesized that there are likely associations between HCY, B12, FA, and ED. In order to comprehensively investigate the exact association between HCY, B12, FA, and ED, our study is conducted based on the Fangchenggang Area Male Health and Examination Survey (FAMHES) project. Our study may thereby pave the way to the treatment of ED on the basis of the balance among HCY, B12, and FA.

Methods and Materials

Population and data collection

FAMHES is a population-based project, which was mainly performed to investigate environmental and genetic factors, as well as their interrelations. 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. Then, 3593 participants responded for further interviews (response rate = 83.5%). [13] No distinct differences were detected between the men who participated in the interviews and those who did not. All participants signed a form indicating that they had provided their informed consent to study participation. This study was approved by the Medical Ethics Committee of Guangxi Medical University.

All participants were asked to provide blood samples between 8:00 am and 11:00 am, after fasting for at least 8 h (overnight). Then, 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. Serum B12 and FA were detected with electrochemiluminescence immunoassays, while serum HCY was measured with enzymatic cycling methods.

Then, all the participants were invited to complete a comprehensive questionnaire. This process was performed by the trained investigators using a standardized protocol with a face-to-face interview. Essential information (e.g., age, sex, smoking, and drinking) was collected, and complete physical examinations (e.g., height, weight, waistline, and hipline) were performed. Smoking status and alcohol consumption were defined as Yes or No. The marital status was classified into living together (married or cohabitation without marriage) and alone (bachelors or bachelorhood). Meanwhile, according to the years of education, three groups could be defined (0-6 years: Primary education; 7-12 years: Intermediate education; and ≥ 13 years: Superior education). In the physical examination, body weight with thin clothing and height without shoes were measured. Then, body mass index (BMI) was calculated with the formula of weight/(height)^2. The waist circumference was measured at the midpoint between the inferior costal margin and the superior iliac crest in the midaxillary line. The hipline was defined as the maximum circumference over the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference/hipline. 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. The flow of participants' collection is shown in **Figure S1**.

Patient and Public Involvement

Patients and the public were not involved in the development of the research question and design or recruitment of this study.

ED definition and grouping

In this study, the International Index of Erectile Function (IIEF-5) was applied to define ED. [14] The IIEF-5 system has five questions, which mainly cover the conditions of erection confidence, erection firmness, maintenance ability, maintenance frequency, and satisfaction, with the scores ranging from 5 to 25. Each question has six selections. According to the orders of answers, the scores are defined as 0–5. Then, participants can be divided into ED (IIEF-5 ≤ 21) and Non-ED (IIEF-5 > 21) groups. According to the symptoms, ED can also be classified into five groups: none (IIEF-5 score 22-25); mild (17-21); moderate (12-16); and severe symptoms (5-11). [13, 15] In addition, HCY level can also be divided into normal (HCY 5-15μmol/L) and hyperhomocysteinemia (HCY > 15μmol/L). [16]

Participants screening

In order to acquire the eligible participants for this study, we developed rigorous exclusion criteria: (i) incomplete data for the individual information and IIEF-5 score; (ii) incomplete data for HCY, B12, and FA or refused to provide the blood samples; (iii) with diseases such as cardiovascular diseases, inflammatory/immune diseases, and kinds of cancers, which might influence the levels of HCY, B12, and FA (**Table S1**); and (iv) currently taking drugs that might affect the HCY, B12, and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine, or glucocorticoids (**Table S1**). Then, 1381 participants were included for further analyses. The flow for screening the eligible participants is shown in **Figure S2**.

Statistical analysis

Before analysis, HCY, B12, and FA levels were tested for Gaussian distribution with the Shapiro-Wilks test. If data were not Gaussian in distribution, they were logarithmically transformed, in order to ensure the approximate Gaussian distribution. Based on the 22 IIEF-5 scores, two groups were defined (ED and Non-ED), and Student's t-test and the chi-squared (X^2) test were applied in the baseline analysis. Then, linear and logistic regression analyses were used, with the IIEF-5 scores and binary variable (ED or Non-ED) as the dependent factors, respectively. Three adjusted models were used: Unadjusted, Age-adjusted, and Multivariate adjusted. In the Multivariate adjusted model, the covariates were as follows: age, smoking status, alcohol consumption, BMI, and WHR. Among them, BMI and WHR are the indexes applied to estimate obesity. However, BMI tends to evaluate body fatness but has a weak ability to differentiate fatness as central or visceral.[17] 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.[17, 18] Additionally, the predictive effects of BMI and WHR in diseases are different.[19, 20] So, in our study, these two obesity indexes were treated as the co-variates.

Then, the multinomial logistic regression analysis was also performed to discover the potential association between HCY, B12, FA, and ED, along with the order of severity of ED or the changes in the HCY, B12, and FA levels quartile (Q1 < 25%, 25% ≤ Q2 ≤ 50%, 50% < Q3 ≤ 75%, and Q4 > 75%). Additionally, considering the non-negligible influences of age on the risk of ED, we also grouped the participants on the basis of age (< 40, 40-49, 50-59, and ≥ 60 years old). The Bernoulli correction was applied, with the significant threshold of $P < 0.0125$ (= 0.05/4 tests) for multinomial logistic regression analysis. Additionally, according to the groups of marital status and educational status, the logistic regression analyses were also conducted. In these analyses, the missing data was deleted. All statistical tests were two-tailed, which were performed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The threshold for significance was $P < 0.05$.

Results

In the baseline analysis, based on IIEF-5, the ED and Non-ED groups were defined. In line with previous studies, the age of the ED group (37.99 ± 10.75 years) was older than the Non-ED group (34.18 ± 8.47 years, $P < 0.001$). Meanwhile, B12 levels were significantly higher in the ED group ($P = 0.015$). Although, no significant difference was shown for HCY levels, the proportion of hyperhomocysteinemia was higher in the ED group (43.02%) than that in the Non-ED group (37.52%, $P = 0.037$). In addition, the proportion of alcohol consumption ($P = 0.032$) and educational status ($P < 0.001$) were also identified to have statistically significant difference in the two groups (**Table 1**).

Signal for the association between HCY and ED

While we discovered no significant association between HCY levels and ED in the comprehensive analyses (**Table 2-5**), a slight association of HCY with ED was observed in the participants grouped by age, especially in the old men (age ≥ 60) (**Table S2**). Similar relevance was confirmed in the marital status (alone, Unadjusted severe ED: OR = 4.385, 95% CI = 1.070-17.974, $P = 0.040$; Age-adjusted severe ED: OR = 5.085, 95% CI = 1.195-21.636, $P = 0.028$) (**Table**

S3).

In the latter analysis, the HCY was divided into normal (HCY 5-15 μmol/L) and hyperhomocysteinemia (HCY > 15 μmol/L). The significant association between HCY and ED seemed to be more prominent in the men living alone (Age-adjusted severe ED: OR = 2.448, 95% CI = 1.046-5.733, P = 0.039) (**Table S3**).

B12 level is significantly associated with ED

To investigate the association between ED and B12, we applied linear and logistic regression analyses, resulting in no significant association for B12 in the linear regression analysis (in which IIEF-5 scores were treated as the dependent factor). For the binary logistic regression (the status of ED evaluated by IIEF-5 was treated as the dependent factor), B12 was identified to be associated with ED in the unadjusted model (OR = 1.438, 95% CI = 1.070-1.933, P = 0.016). However, the association signal diminished in other adjusted models (**Table 3**). We next investigated the relationship between B12 and ED, based on the severity grades of ED. Interestingly, the positive correlation between B12 and ED was further confirmed, especially among men 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**). Subsequently, the levels of B12 were divided into quartiles. The result showed that B12 might be significantly associated with 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; and P for trend < 0.001) (**Table 5**).

After adjusting age for the above analyses, the significant association between B12 and ED diminished (**Table 2 and 3** and **Table 5**), suggesting that age cannot be excluded while investigating the relationship between B12 and ED. We thus grouped the participants into four age groups (ages < 40, 40-49, 50-59, and ≥ 60 years old). Our results showed that the significant correlations between B12 and mild ED (IIEF-5 = 17–21) mainly presented in the 40–49 years old age group (OR = 2.907, 95% CI = 1.402-6.026, P = 0.004) (**Table S2**).

Our baseline analysis discovered different proportions of educational status in the ED and Non-ED groups. In order to discuss the influences of marital and educational status in the relevance to ED and B12, we further performed between-group analyses. Similar to previous results, B12 was also identified to be associated with mild ED, even after multivariate adjustment (marital status, living together: OR = 1.501, 95% CI = 1.035-2.175, P = 0.032; alone: OR = 3.449, 95% CI = 1.113-10.692, P = 0.032; and educational status, Intermediate: OR = 1.858, 95% CI = 1.214-2.845, P = 0.004) (**Table S3**).

Discussion

ED is a common disorder, affecting a large number of males. [1–4] Recent studies suggest HCY may be an independent risk factor for ED. [8, 9] In order to test this association, we conducted current study based on the larger population-based FAMHES project. We confirmed that HCY is significantly associated with ED, especially severe ED. Moreover, B12 may also be relevant to mild ED. In contrast, we observed no significant association between FA and ED in our study.

HCY was reported to be associated with many diseases and health conditions, such as psychological disorders, [21, 22] lipid profiles, [23] renal Impairment, [24] and inflammatory/immune

factors. [25] Moreover, HCY is also identified to be a useful marker for CVD. [26, 27] Meanwhile, ED could be a potentially predictive factor for cardiovascular and other chronic diseases. [28] Based on the relevance, it was assumed that HCY might be a risk factor for ED. [8, 9] In consistent with this, we revealed that HCY was significantly associated with ED, especially severe ED. The main mechanism might be that HCY could influence endothelial dysfunction and nitric oxide (NO) diffusion. Previously, in vitro and in vivo studies discovered that HCY could be a toxin for the vasculature by inducing endothelial dysfunction. [29] Additionally, NO is mainly involved in vascular dilation, smooth muscle relaxation (including genitourinary smooth muscle), and permitting penile erection. [30, 31] Studies showed that increased HCY could inhibit NO synthase, thereby probably influencing the production of NO, and the development of ED. [32] So, on the basis of these relevance, we could understand the risk effect of HCY on ED. Additionally, the status of living alone for men would also influence this association, hinting the pathogenesis of psychological factors for ED.

B12 is also known as cobalamin. Similar to FA, it is an important cofactor in methionine synthesis and homocysteine metabolism. [33] Although previous studies identified that FA might be a potential protective factor for ED, [34] no significant association has been detected. In contrast to B12, HCY has been found to protect against ED. [35] Our study also identifies the potential association between B12 and ED, though 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 prominent for mild ED at the higher B12 levels. There are two possible explanations. First, our results suggest that the function of B12 in ED might be dose-dependent. Excessive 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, defense mechanisms are triggered. As a potential protective factor, the absorption of B12 is enhanced. Combining the limited reports, 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.

Limitations

Our study verified the previous conclusions that HCY could increase the risk of ED. However, some limitations still need to be noted: (i) this study is a cross-sectional analysis, which just reflects the status of specific time points and populations; (ii) there are limited numbers of participants with primary educational status. So, the results need to be examined further; (iii) although we have identified a significant association between B12 and ED, the exact mechanisms and effects were unclear until now; and (iv) after multiple testing, no positive association can be detected, suggesting that our results might be unstable. So, further studies will be needed.

Conclusions

ED is one of the most common male diseases. This study was conducted in order to discover the functions of HCY, B12, and FA in ED. Our results confirmed the pathogenic effect of HCY on ED, especially on severe ED. Meanwhile, B12 was likely to be significantly associated with ED. Further studies with larger cohorts of participants should be focused on the potential

mechanisms and therapeutic effects of B12 on ED.

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Conflict of Interest

There are no conflicts of interests.

Author Contributions

Y.C., J.L., Z.N.M., and J.W.C. participated in participants' collection, field investigation, design, writing and modification of all the paper. Y.C. and J.L. took part in the statistical analysis. Z.N.M. and J.W.C. provided important advices for this paper. T.Y.L., J.X.L., J.L.L. and G.H.W. provide efforts in the processes of modification.

Data Sharing Statement

The data for this study was available in the supplementary materials. Further questions could be sent to ZN.M (zengnanmo@hotmail.com) and JW.C (chengjiwen1977@foxmail.com).

References

1. Braun M, Wassmer G, Klotz T, et al. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res 2000;12:305-11. [PubMed: 11416833]
2. Pinnock CB, Stapleton AM, Marshall VR. Erectile dysfunction in the community: a prevalence study. Med J Aust 1999;171:353-7. [PubMed: 10590723]
3. Nicolosi A, Moreira ED Jr, Shirai M, et al. Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectile dysfunction. Urology 2003;61:201-6. [PubMed: 10590723]
4. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 1999;84:50-56. [PubMed: 10444124]
5. Kloner RA, Speakman M. Erectile dysfunction and atherosclerosis. Curr Atheroscler Rep 2002;4:397-401. [PubMed: 12162940]
6. Sullivan ME, Keoghane SR, Miller MA. Vascular risk factors and erectile dysfunction. BJU Int 2001;87:838-45. [PubMed: 11412223]
7. Nehra A, Jackson G, Miner M, et al. Diagnosis and treatment of erectile dysfunction for reduction of cardiovascular risk. J Urol 2013;189:2031-8. [PubMed: 23313195]
8. 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. [PubMed: 10510122]

9. 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. [PubMed: 27709655]
10. Long Y, Nie J. Homocysteine in Renal Injury. Kidney Dis (Basel) 2016; 2:80-7. [PubMed: 27536696]
11. Lai WK, Kan MY. Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1-12. [PubMed: 26201664]
12. Sansone M, Sansone A, Romano M, et al. Folate: a possible role in erectile dysfunction? Aging Male. 2017 Nov 20:1-5. [PubMed: 29157083]
13. 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. [PubMed: 25343742]
14. Rosen RC, Cappelleri JC, Smith MD, et al. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res 1999;11:319-26. [PubMed: 10637462]
15. Kupelian V, Araujo AB, Chiu GR, et al. Relative contributions of modifiable risk factors to erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. Prev Med 2010;50:19-25. [PubMed: 19944117]
16. Guo H, Chi J, Xing Y, et al. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res 2009; 129:279-84. [PubMed: 19491420]
17. McDonnold M, Mele LM, Myatt L, et al. Waist-to-Hip Ratio versus Body Mass Index as Predictor of Obesity-Related Pregnancy Outcomes. Am J Perinatol. 2016;33:618-624. [PubMed: 26788786]
18. Suchanek P, Kralova Lesna I, et al. Which index best correlates with body fat mass: BAI, BMI, waist or WHR? Neuro Endocrinol Lett. 2012;33 Suppl 2:78-82. [PubMed: 23183515]
19. Tang B, Han CT, Zhang GM, et al. 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;7:43551. [PubMed: 28272469]
20. Welborn TA, Dhaliwal SS. Preferred clinical measures of central obesity for predicting mortality. Eur J Clin Nutr. 2007;61:1373-9. [PubMed: 17299478]
21. Salagre E, Vizuete AF, Leite M, et al. Homocysteine as a peripheral biomarker in bipolar disorder: A meta-analysis. Eur Psychiatry. 2017;43:81-91. [PubMed: 28371745]
22. Elstgeest LE, Brouwer IA, Penninx BW, et al. Vitamin B12, homocysteine and depressive symptoms: a longitudinal study among older adults. Eur J Clin Nutr 2017;71:468-75. [PubMed: 28145420]
23. Momin M, Jia J, Fan F, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis 2017; 16:54. [PubMed: 28288621]
24. Chen J, Li G, Xu Z, et al. Elevated Plasma Homocysteine Level Increased the Risk of Early Renal Impairment in Acute Ischemic Stroke Patients. Cell Mol Neurobiol 2017;37:1399-1405. [PubMed: 28275883]
25. Li T, Chen Y, Li J, et al. Serum Homocysteine Concentration Is Significantly Associated with Inflammatory/Immune Factors. PLoS One 2015; 10:e0138099. [PubMed: 26367537]
26. Sahu A, Gupta T, Kavishwar A, et al. Cardiovascular Diseases Risk Prediction by

Homocysteine in Comparison to other Markers: A Study from Madhya Pradesh. J Assoc Physicians India 2015;63:37-40. [PubMed: 27608690]

27. Yeh JK, Chen CC, Hsieh MJ, et al. Impact of Homocysteine Level on Long-term Cardiovascular Outcomes in Patients after Coronary Artery Stenting. J Atheroscler Thromb 2017;24:696-705. [PubMed: 27803490]

28. Baumann F, Hehli D, Makaloski V, et al. Erectile dysfunction - overview from a cardiovascular perspective. Vasa 2017; 1-7. [PubMed: 28486869]

29. McDowell IF, Lang D. Homocysteine and endothelial dysfuncton: A link with cardiovascular disease. J Nutr 2000;130: 369S-372S. [PubMed: 10721909]

30. Rajfer J, Aronson WJ, Bush PA, et al. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Engl J Med 1992;326:90-94. [PubMed: 1309211]

31. Deanfield J, Donald A, Ferri C, et al. Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension: Endothelial function and dysfunction. Part I: methodological issues for assessment in the different vascular beds: a statement by the working group on endothelin and endothelial factors of the European society of hypertension. J Hypertens 2005;23:7-17. [PubMed: 15643116]

32. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-375. [PubMed: 10475890]

33. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2:299-316. [PubMed: 22254022]

34. Yan WJ, Yu N, Yin TL, et al. A new potential risk factor in patients with erectile dysfunction and premature ejaculation: folate deficiency. Asian J Androl 2014;16:902-906. [PubMed: 25080932]

35. Giovannone R, Busetto GM, Antonini G, et al. Hyperhomocysteinemia as an Early Predictor of Erectile Dysfunction: International Index of Erectile Function (IIEF) and Penile Doppler Ultrasound Correlation With Plasma Levels of Homocysteine. Medicine (Baltimore) 2015;94:e1556. [PubMed: 26426624]

	ED	Non-ED	P
No.	688	693	
Age, years	**37.99±10.75**	**34.18±8.47**	**<0.001[b]**
BMI, Kg/m^2	23.27±3.26	23.37±3.48	0.591[b]
WHR	0.88±0.06	0.88±0.06	0.253[b]
HCY, μmol/L	14.97±4.11	15.34±11.09	0.524[b]
Normal HCY	392 (56.98%)	433 (62.48%)	
Hyperhomocysteinemia	**296 (43.02%)**	**260 (37.52%)**	**0.037[b]**
B12, pg/ml	**718.53±234.37**	**688.74±229.68**	**0.015[b]**
FA, ng/ml	9.56±2.72	9.89±11.28	0.594[b]
Smoke			
Yes	392 (56.98%)	385 (55.56%)	
No	296 (43.02%)	308 (44.44%)	0.594[c]
Drink			
Yes	**586 (85.17%)**	**617 (89.03%)**	
No	**102 (14.83%)**	**76 (10.97%)**	**0.032[c]**
Marital status[e]			
Live together	595 (86.48%)	578 (83.41%)	
Alone	93 (13.52%)	115 (16.59%)	0.110[c]
educational status[a]			
Primary	**19 (2.76%)**	**5 (0.72%)**	
Intermediate	**488 (70.93%)**	**411 (59.39%)**	
Superior	**181 (26.31%)**	**276 (39.89%)**	**<0.001[c]**

Table 1. The characteristics of the eligible participants in the analysis

a. One participant without the information of educational status in the Non-ED group

b. Student's t-test

c. chi-square test

e. The marital status was classified into live together (married or cohabitation without marriage) and alone (bachelors or bachelorhood).

* Normal HCY: 5-15μmol/L; hyperhomocysteinemia: >15umol/L

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

	Unadjusted			Age-adjusted			Multivariate adjusted		
	BETA	95%CI	P	BETA	95%CI	P	BETA	95%CI	P
IIEF-5									
HCY	-0.202	-1.080, 0.676	0.651	0.139	-0.732, 1.009	0.755	0.084	-0.787, 0.956	0.850
Binary HCY	-0.338	-0.817, 0.142	0.167	-0.146	-0.622, 0.330	0.548	-0.186	-0.663, 0.291	0.444
B12	0.048	-0.600, 0.696	0.885	0.212	-0.428, 0.852	0.515	0.404	-0.259, 1.068	0.232
FA	0.112	-0.668, 0.891	0.779	0.388	-0.384, 1.160	0.986	0.324	-0.496, 1.145	0.438

Table 2. The linear regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* IIEF-5 scores were the dependent factor for the linear regression analysis.

	Unadjusted			Age-adjusted			Multivariate adjusted		
Binary	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY	1.137	0.766-1.687	0.524	0.959	0.641-1.435	0.839	0.986	0.658-1.479	0.986
Binary HCY	**1.258**	**1.014-1.560**	**0.037**	1.151	0.923-1.435	0.212	1.174	0.940-1.466	0.157
B12	**1.438**	**1.070-1.933**	**0.016**	1.338	0.992-1.805	0.057	1.311	0.961-1.788	0.087
FA	1.100	0.775-1.561	0.594	0.956	0.668-1.367	0.804	1.041	0.710-1.527	0.835

Table 3. The binary regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* In the binary regression analysis, the ED status (ED: IIEF-5≤21; Non-ED: IIEF-5>21) was treated as the dependent factor.

	HCY			Binary_HCY			B12			FA		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
ED-Unadjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	1.081	0.695-1.681	0.730	1.246	0.980-1.583	0.072	**1.694**	**1.207-2.376**	**0.002**	1.180	0.801-1.740	0.402
Moderate (12–16)	1.258	0.649-2.439	0.497	1.298	0.896-1.880	0.168	1.187	0.715-1.972	0.508	0.960	0.519-1.776	0.896
Severe (5–11)	1.259	0.562-2.820	0.575	1.258	0.799-1.980	0.322	0.891	0.496-1.599	0.698	0.923	0.434-1.963	0.834
ED-age-adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.948	0.607-1.480	0.814	1.166	0.914-1.486	0.217	**1.596**	**1.135-2.244**	**0.007**	1.052	0.710-1.558	0.800
Moderate (12–16)	0.929	0.455-1.898	0.840	1.095	0.747-1.605	0.643	1.409	0.635-1.733	0.851	0.762	0.404-1.437	0.401
Severe (5–11)	1.068	0.467-2.443	0.876	1.150	0.726-1.820	0.552	0.839	0.471-1.495	0.551	0.794	0.370-1.705	0.554
ED- Multivariate adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.973	0.621-1.524	0.904	1.188	0.929-1.517	0.169	**1.620**	**1.141-2.300**	**0.007**	1.184	0.775-1.808	0.435
Moderate (12–16)	0.965	0.472-1.971	0.922	1.119	0.762-1.643	0.567	0.972	0.576-1.640	0.915	0.777	0.401-1.507	0.456
Severe (5–11)	1.132	0.491-2.613	0.771	1.187	0.748-1.885	0.467	0.717	0.388-1.324	0.288	0.821	0.368-1.831	0.631

Table 4. Multinomial logistic regression for the association between ED and HCY, B12 and FA

* The categorical dependent variables were the various ED groups, based on the IIEF-5. The symptoms of ED were divided into None (IIEF-5= 22-25), Mild (17-21), Moderate (12-16) and Severe (5-11). And the None group (22-25) was treated as the reference.

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

	Unadjusted			Age-adjusted			Multivariate adjusted		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.130	0.838-1.525	0.422	1.022	0.753-1.387	0.887	1.012	0.744-1.377	0.938
Q3	1.292	0.958-1.743	0.094	1.187	0.875-1.610	0.271	1.210	0.890-1.646	0.224
Q4	1.276	0.946-1.720	0.110	1.091	0.803-1.483	0.578	1.103	0.810-1.502	0.534
B12									
Q1	1	1	1	1	1	1	1	1	1
Q2	0.917	0.680-1.236	0.569	0.893	0.659-1.209	0.464	0.899	0.662-1.221	0.496
Q3	0.988	0.733-1.333	0.939	0.972	0.717-1.316	0.853	0.986	0.726-1.338	0.927
Q4	**1.452**	**1.076-1.961**	**0.015**	1.299	0.955-1.765	0.095	1.286	0.945-1.752	0.110
FA									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.313	0.974-1.770	0.074	1.325	0.978-1.795	0.069	1.332	0.981-1.811	0.067
Q3	1.300	0.963-1.755	0.086	1.243	0.916-1.687	0.163	1.286	0.944-1.751	0.111
Q4	1.198	0.888-1.616	0.236	1.094	0.806-1.487	0.564	1.116	0.819-1.522	0.487

Table 5. Association between HCY, B12, FA and ED along with the increased levels of these indexes

* In the Multinomial logistic regression, the levels of HCY, B12, and FA was divided into quartile (Q1<levels of 25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%), 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".

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

Excluded diseases	Excluded drugs
Hypertension	Vitamins: Vitamins B12, Vitamins C, Vitamins B2, etc.
Diabetes	Antidiabetic medicines: insulin, metformin, Ningestedglinide, etc.
Angina	Non-steroidal anti-inflammatory drugs: aspirin, acetaminophen, indomethacin, diclofenac, celecoxib, etc.
Myocardial infarction	Antibiotics: penicillin, cephalosporins, aztreonam, ofloxacin, clarithromycin, etc.
Stroke	Cimetidine:
Gout	Glucocorticoids: hydrocortisone, prednisone, methylprednisolone, hexadecadrol, etc.
Rheumatoid arthritis	
Prostatitis	
Hepatitis B	
Various cancers	
Parotiditis	
Urolithiasis	
Pelvic floor surgery	

Table S1. The list of diseases and drugs excluded in this study

		No. ED	No. Non-ED	HCY			Binary HCY			B12			FA		
				BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P
Ages <40															
IIEF-5		409	531	-0.246	-1.32, 0.837	0.656	-0.269	-0.845, 0.307	0.359	0.627	-0.202, 1.456	0.138	-0.137	-1.135, 0.862	0.788
ED				0.987	0.594-1.639	0.960	1.147	0.876-1.501	0.319	1.207	0.818-1.780	0.344	1.146	0.718-1.829	0.567
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.961	0.547-1.688	0.889	1.185	0.881-1.594	0.263	1.460	0.945-2.255	0.088	1.162	0.692-1.950	0.571
	Moderate			0.794	0.282-2.238	0.662	0.843	0.486-1.462	0.544	0.957	0.444-2.066	0.912	1.015	0.401-2.568	0.974
	Severe			1.429	0.469-4.357	0.530	1.351	0.732-2.495	0.336	0.544	0.223-1.326	0.180	1.276	0.432-3.766	0.659
40-49		176	131												
IIEF-5				0.501	-1.025, 2.207	0.519	-0.109	-1.060, 0.841	0.821	0.320	-0.904, 1.544	0.607	0.990	-0.622, 2.602	0.228
ED				0.842	0.398,-1.778	0.651	1.129	0.710-1.795	0.608	1.692	0.925-3.094	0.088	0.878	0.398-1.937	0.747
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.820	0.358-1.878	0.638	1.135	0.685-1.882	0.623	**2.907**	**1.402-6.026**	**0.004**	1.174	0.495-2.785	0.716
	Moderate			0.951	0.288-3.146	0.935	1.267	0.598-2.682	0.537	0.550	0.224-1.355	0.194	0.380	0.102-1.407	0.147
	Severe			0.845	0.140-5.123	0.855	0.905	0.318-2.578	0.852	0.910	0.243-3.403	0.889	0.579	0.105-3.183	0.530
50-59		69	21												
IIEF-5				4.297	-0.514, 9.108	0.079	1.597	-0.408, 3.602	0.117	-2.046	-4.862, 0.770	0.152	-1.649	-5.387, 2.088	0.383
ED				0.314	0.028-3.584	0.351	0.867	0.309-2.430	0.786	1.521	0.355-6.516	0.572	1.773	0.265-11.867	0.555
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.379	0.024-5.901	0.489	0.927	0.299-2.875	0.895	0.859	0.171-4.308	0.853	1.601	0.198-12.945	0.659
	Moderate			0.669	0.030-14.774	0.799	1.326	0.356-4.931	0.674	4.335	0.603-31.187	0.145	1.042	0.086-12.647	0.974
	Severe			0.046	0.001-1.941	0.107	0.358	0.077-1.660	0.189	2.060	0.256-16.573	0.497	3.751	0.263-53.516	0.330
≥60		34	10												
IIEF-5				-0.479	-7.400, 6.442	0.889	-0.116	-3.491, 3.259	0.945	-0.526	-4.308, 3.255	0.779	2.899	-2.726, 8.523	0.303
ED				29.170	0.379-2.243E3	0.128	2.544	0.409-15.822	0.317	0.714	0.089-5.723	0.751	0.523	0.018-15.079	0.705
	None			1	1	1	1	1	1	1	1	1	1	1	1

	Mild	**767.519**	**1.649-3.573E5**	**0.034**	4.093	0.337-49.760	0.269	0.677	0.038-12.139	0.791	0.645	0.008-52.675	0.845
	Moderate	11.236	0.098-1.289E3	0.317	2.266	0.283-18.119	0.441	1.067	0.092-12.408	0.959	2.717	0.049-150.689	0.626
	Severe	70.920	0.291-1.729E4	0.129	3.281	0.316-34.092	0.320	0.424	0.037-4.866	0.491	0.027	0.000-3.577	0.148

Table S2. Association between ED and HCY, B12, FA level in four ages groups (<40, 40-49, 50-59 and ≥60 years) after adjusting multivariate

* Linear regression for the IIEF-5; binary regression analysis for ED; multinomial logistic regression for the severity of symptom of ED

* ED is divided into four groups according to the IIEF-5 scores: None=22-25 scores, Mild=17-21 scores, Moderate=12-16 scores and Severe=5-11 scores. None ED is treated as the reference.

* ED: erectile dysfunction; HCY= homocysteine; B12= vitamin B12; FA= folic acid; BMI: body mass index; WHR: waist hip rate; OR: odd ratio; 95%CI: 95% confidence interval

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* Multi-adjusted: age, BMI, WHR, smoke and drink

	ED grading	HCY			Binary_HCY			B12			FA		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Unadjusted													
Marital status													
Live together		1.070	0.698-1.640	0.757	1.237	0.979-1.562	0.074	**1.400**	**1.010-1.940**	**0.044**	1.283	0.860-1.916	0.222
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.077	0.673-1.722	0.758	1.239	0.959-1.601	0.101	**1.484**	**1.033-2.132**	**0.033**	1.295	0.833-2.012	0.251
	Moderate	1.267	0.629-2.555	0.508	1.367	0.920-2.032	0.122	1.105	0.636-1.921	0.723	1.158	0.583-2.303	0.675
	Severe	0.678	0.227-2.027	0.487	0.987	0.566-1.722	0.964	1.534	0.697-3.375	0.288	1.492	0.580-3.841	0.407
Alone		1.523	0.540-4.299	0.426	1.346	0.765-2.370	0.303	1.458	0.705-3.013	0.309	0.577	0.246-1.356	0.207
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.813	0.207-3.194	0.767	1.169	0.580-2.355	0.662	**3.950**	**1.331-11.719**	**0.013**	0.750	0.285-1.976	0.561
	Moderate	0.985	0.128-7.560	0.988	0.812	0.267-2.470	0.714	1.568	0.352-6.975	0.555	0.330	0.058-1.878	0.211
	Severe	**4.385**	**1.070-17.974**	**0.040**	2.249	0.974-5.193	0.058	0.605	0.252-1.450	0.260	0.460	0.116-1.824	0.269
educational status													
Primary		1.586	0.018-142.652	0.841	0.875	0.116-6.579	0.897	5.169	0.188-142.118	0.331	0.399	0.095-1.676	0.209
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	3.257	0.018-596.353	0.657	0.500	0.045-5.514	0.571	4.007	0.136-117.975	0.421	0.455	0.088-2.364	0.349
	Moderate	0.489	0.002-114.662	0.797	1.500	0.136-16.542	0.741	**1.266E^{3}**	**2.303-6.962E^{5}**	**0.026**	0.423	0.060-2.975	0.387
	Severe	2.199	0.007-686.953	0.788	1.000	0.080-12.557	1.000	2.627	0.200-34.591	0.463	0.205	0.009-4.472	0.314
Intermediate		1.667	0.989-2.811	0.055	1.305	0.993-1.716	0.056	**1.513**	**1.049-2.182**	**0.027**	1.187	0.747-1.887	0.469
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.571	0.883-2.795	0.124	1.295	0.958-1.752	0.093	**1.838**	**1.215-2.781**	**0.004**	1.304	0.779-2.181	0.312
	Moderate	1.619	0.692-3.785	0.266	1.265	0.806-1.986	0.307	1.132	0.620-2.067	0.687	0.888	0.411-1.919	0.763
	Severe	2.355	0.876-6.326	0.089	1.429	0.829-2.463	0.199	0.934	0.452-1.926	0.852	1.160	0.451-2.980	0.759
Superior		0.874	0.449-1.699	0.691	1.436	0.986-2.093	0.060	0.938	0.537-1.640	0.823	1.117	0.586-2.129	0.738
	None	1	1	1	1	1	1	1	1	1	1	1	1

	Mild	0.765	0.351-1.669	0.501	1.403	0.924-2.130	0.112	1.156	0.612-2.183	0.655	1.202	0.587-2.462	0.615
	Moderate	1.581	0.567-4.414	0.381	1.800	0.849-3.818	0.126	0.640	0.225-1.821	0.403	1.244	0.345-4.481	0.739
	Severe	0.479	0.064-3.567	0.472	1.170	0.461-2.970	0.741	0.491	0.142-1.691	0.259	0.569	0.116-2.788	0.487
Age-Adjusted													
Marital status													
Live together		9.868	0.559-1.347	0.528	1.110	0.873-1.412	0.394	1.333	0.954-1.861	0.092	1.122	0.743-1.694	0.584
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.940	0.586-1.508	0.796	1.157	0.892-1.501	0.271	1.436	0.996-2.072	0.053	1.176	0.752-1.839	0.478
	Moderate	0.874	0.402-1.902	0.735	1.110	0.735-1.676	0.620	1.015	0.583-1.766	0.958	0.947	0.469-1.909	0.878
	Severe	0.378	0.114-1.258	0.113	0.762	0.428-1.357	0.356	1.353	0.622-2.943	0.445	1.193	0.459-3.097	0.717
Alone		1.611	0.566-4.586	0.371	1.406	0.793-2.493	0.243	1.391	0.653-2.961	0.392	0.622	0.257-1.506	0.292
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.216-3.352	0.817	1.210	0.598-2.451	0.596	**3.742**	**1.258-11.129**	**0.018**	0.799	0.294-2.169	0.659
	Moderate	1.019	0.132-7.836	0.986	0.831	0.272-2.542	0.745	1.492	0.339-6.562	0.597	0.341	0.060-1.947	0.226
	Severe	**5.085**	**1.195-21.636**	**0.028**	**2.448**	**1.046-5.733**	**0.039**	0.450	0.175-1.159	0.098	0.516	0.122-2.174	0.367
educational status													
Primary		0.285	0.001-61.236	0.647	0.212	0.010-4.431	0.317	10.044	0.027-3.674E^{3}	0.444	0.421	0.080-2.228	0.309
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.588	0.004-601.127	0.879	0.228	0.010-5.326	0.357	4.021	0.062-259.697	0.513	0.485	0.093-2.542	0.392
	Moderate	0.001	7.773E^{-8}-6.096	0.116	0.139	0.003-6.581	0.316	3.874E^{3}	1.164-1.289E^{7}	0.046	0.426	0.024-7.587	0.561
	Severe	0.035	1.430E^{-5}-84.089	0.398	0.118	0.002-6.145	0.290	8.466	0.008-8.474E^{3}	0.545	0.162	0.005-5.514	0.311
Intermediate		1.392	0.820-2.365	0.221	1.209	0.915-1.597	0.182	1.384	0.954-2.009	0.087	1.073	0.669-1.722	0.769
	None	1	1	1	1	1		1	1	1	1	1	1
	Mild	1.382	0.774-2.468	0.275	1.229	0.906-1.668	0.186	**1.723**	**1.133-2.620**	**0.011**	1.206	0.717-2.030	0.480
	Moderate	1.145	0.470-2.787	0.766	1.084	0.682-1.724	0.733	0.966	0.529-1.764	0.911	0.754	0.346-1.643	0.478
	Severe	1.915	0.698-5.257	0.207	1.298	0.748-2.254	0.354	0.840	0.409-1.726	0.635	1.022	0.397-2.632	0.964
Superior		0.779	0.391-1.552	0.477	1.324	0.900-1.948	0.154	0.953	0.540-1.684	0.869	0.898	0.461-1.750	0.751

	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.672	0.297-1.521	0.340	1.289	0.841-1.976	0.245	1.175	0.618-2.238	0.623	0.960	0.458-2.012	0.914
	Moderate	1.421	0.486-4.155	0.521	1.583	0.736-3.405	0.240	0.682	0.241-1.926	0.470	0.917	0.244-3.440	0.898
	Severe	0.521	0.074-3.647	0.511	1.202	0.472-3.061	0.699	0.473	0.132-1.690	0.249	0.622	0.125-3.096	0.562
Multivariate adjusted													
Marital status													
Live together		0.894	0.575-1.390	0.619	1.137	0.893-1.448	0.299	1.349	0.962-1.891	0.083	1.176	0.773-1.789	0.449
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.963	0.598-1.548	0.875	1.181	0.909-1.534	0.213	**1.501**	**1.035-2.175**	**0.032**	1.245	0.790-1.961	0.345
	Moderate	0.913	0.420-1.985	0.818	1.136	0.751-1.719	0.547	0.971	0.554-1.701	0.918	0.933	0.455-1.915	0.851
	Severe	0.417	0.126-1.378	0.152	0.812	0.454-1.453	0.483	1.227	0.562-2.675	0.608	1.317	0.494-3.514	0.582
Alone		1.602	0.546-4.698	0.390	1.399	0.779-2.512	0.261	1.288	0.558-2.975	0.553	0.678	0.253-1.820	0.441
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.206-3.498	0.821	1.199	0.579-2.481	0.625	**3.449**	**1.113-10.692**	**0.032**	0.959	0.279-3.292	0.947
	Moderate	1.130	0.135-9.481	0.910	0.856	0.276-2.654	0.788	1.244	0.261-5.929	0.784	0.336	0.054-2.069	0.239
	Severe	4.181	0.951-19.374	0.058	2.356	0.989-5.616	0.053	0.302	0.084-1.083	0.066	0.598	0.139-2.570	0.490
educational status													
Primary		0.045	0.000-51.900	0.388	0.098	0.002-5.651	0.262	6.248	0.006-6.418E3	0.605	2.823	0.032-247.907	0.649
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.198	$4.915E^{-5}$-794.766	0.702	0.224	0.004-13.29	0.473	0.001	3.247E-10-3.819E3	0.376	1.806	0.007-479.698	0.836
	Moderate	$2.184E^{-12}$	$2.988E^{-28}$-$1.597E^{4}$	0.150	0.004	1.429E-6-8.838	0.157	**NA**	**NA**	**0.043**	3.396	$0.006\text{-}1.820E^{3}$	0.703
	Severe	1.972E-9	$1.318E^{-24}$-$2.952E^{6}$	0.261	0.008	$4.142E^{-6}$-15.343	0.210	**$8.065E^{-217}$**	**0.000-0.203**	**0.049**	0.516	0.001-457.324	0.849
Intermediate		1.431	0.842-2.432	0.186	1.223	0.925-1.618	0.158	1.428	0.981-2.081	0.063	1.092	0.677-1.764	0.717
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.401	0.782-2.510	0.257	1.238	0.910-1.682	0.174	**1.858**	**1.214-2.845**	**0.004**	1.268	0.748-2.149	0.378
	Moderate	1.225	0.502-2.990	0.656	1.105	0.694-1.761	0.673	0.954	0.519-1.755	0.880	0.707	0.319-1.568	0.394
	Severe	2.044	0.744-5.615	0.165	1.350	0.776-2.351	0.288	0.754	0.364-1.562	0.448	0.969	0.367-2.562	0.950

Superior		0.812	0.405-1.627	0.557	1.360	0.920-2.011	0.123	0.977	0.550-1.738	0.938	0.889	0.449-1.762	0.737
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.702	0.308-1.598	0.399	1.325	0.859-2.044	0.204	1.202	0.632-2.287	0.575	0.898	0.419-1.926	0.783
	Moderate	1.437	0.483-4.270	0.514	1.556	0.718-3.370	0.262	0.647	0.217-1.930	0.435	1.056	0.274-4.063	0.937
	Severe	0.598	0.092-3.890	0.591	1.333	0.513-3.459	0.555	0.496	0.123-2.002	0.325	0.715	0.145-3.529	0.681

Table S3. The logistic regression analyses (binary and multinomial) for the association between ED and HCY, B12 and FA in the subgroups of marital status and educational status

* In the educational status, the Primary group only contains 24 participants. So, some results of regression analyses were exaggerated with these limited data

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15µmol/L); hyperhomocysteinemia (>15umol/L)

* ED status: none (IIEF-5 score 22–25); mild (17–21); moderate (12–16); and severe (5–11).

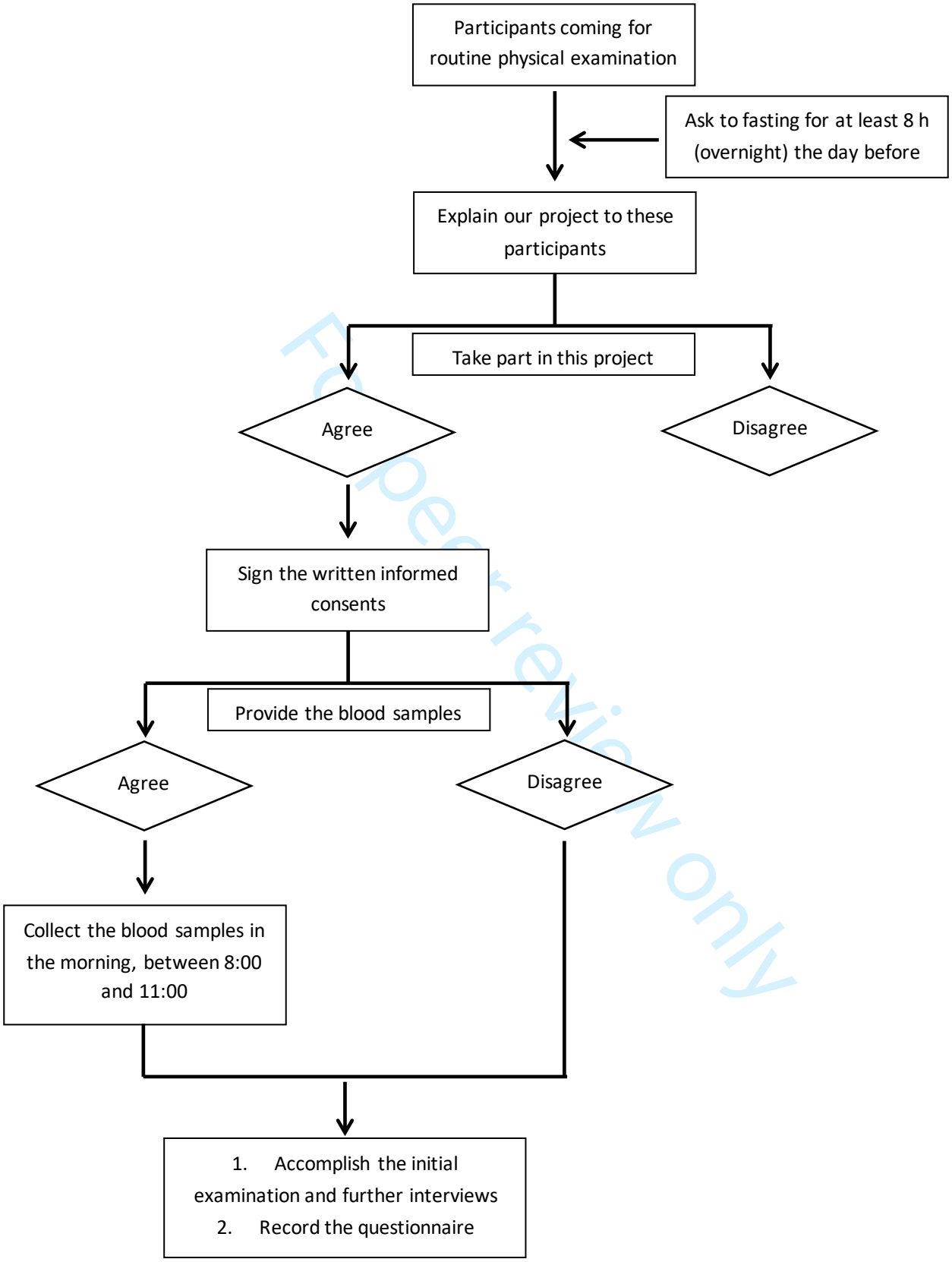

Figure S1. The flow of participants' collection

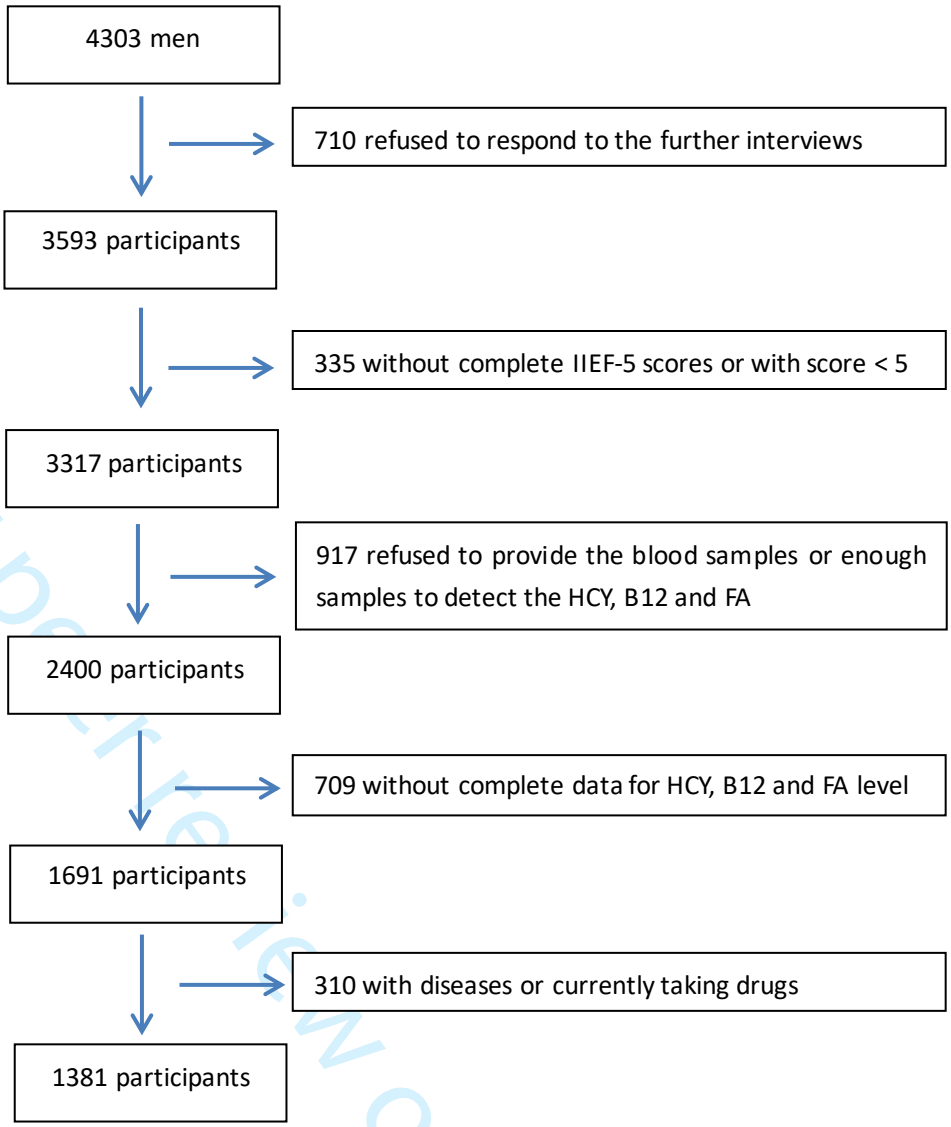

Figure S2. The flow for screening the eligible participants

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	*4*
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6
		(*b*) Report category boundaries when continuous variables were categorized	5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study in China

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Association between homocysteine, vitamin B12, folic acid, and erectile dysfunction: a cross-sectional study in China

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Abstract

Objectives: Erectile dysfunction (ED) affects up to 53.4% of men aged 30-80 years. In this study, we aimed to examine the association between homocysteine (HCY), vitamin B12 (B12), folic acid (FA), and ED.

Design: Cross-sectional study.

Setting: Guangxi, China.

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.

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 (bachelors or bachelorhood). 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.

Keywords: erectile dysfunction, homocysteine, vitamin B12, folic acid

Strengths and limitations of this study

1. Our study is a cross-sectional study, mainly based on the large Fangchenggang Area Male Health and Examination Survey (FAMHES) project, including a total of 4303 men.
2. This study includes comprehensive analyses of baseline, linear and logistic regression, and multinomial logistic regression.
3. According to the changes in the HCY, B12, and FA levels, and the order of ED severity, we investigated the associations between HCY, B12, FA, and ED.
4. The study also took into consideration of the effects of age, marital and educational status.
5. Nevertheless, as a cross-sectional analysis, the exact mechanisms of the relationship between HCY, B12, FA, and ED cannot be clearly defined.

Introduction

Erectile dysfunction (ED) is defined as the inability to acquire and maintain satisfying sexual intercourse with a sufficient erection, affecting up to 53.4% of men aged 30 to 80 years. [1] The morbidity increases sharply among men over 40 years of age. [2, 3] It has been estimated that the prevalence of ED will reach 322 million worldwide by the year 2025. [4]

Various factors including smoking, hypertension, and hyperlipidemia have been identified to influence the development of ED. Among these factors, the vascular component is dominant. [5, 6] Moreover, ED may be one of the indicators of cardiovascular disease (CVD). [7] Homocysteine (HCY) , a CVD-associated factor was recently defined as an independent risk factor for ED. [8, 9] HCY is a thiol-containing amino acid, mainly from methionine, with two steps of transformation. First, methionine is catalyzed to form S-adenosylmethionine (SAM) by the enzyme SAM synthase. As a major methyl group donor for various methylation reactions, SAM is mainly transformed into S-adenosylhomocysteine (SAH) after loss of the methyl group. In the second step, SAH is hydrolyzed by SAH hydrolase to form HCY and adenosine. Biologically, HCY is involved in two pathways, including remethylation (RM) and transsulfuration (TS). In the RM pathway, HCY regenerates methionine by methylenetetrahydrofolate reductase (MTHFR) with cofactors of folic acid (FA) and vitamin B12 (B12). In the TS pathway, HCY is catalyzed by the cystathione-β-synthase (CBS) and γ-cystathionase. [10, 11]

FA and B12 as the cofactors of HCY, have also been identified to be associated with ED. [12] However, limited studies have been focused on the relevance of their levels to ED. On the basis of previous studies, we hypothesized that there are likely associations between HCY, B12, FA, and ED. In order to comprehensively investigate the exact association between HCY, B12, FA, and ED, our study is conducted based on the Fangchenggang Area Male Health and Examination Survey (FAMHES) project. Our study may thereby pave the way to the treatment of ED on the basis of the balance among HCY, B12, and FA.

Methods and Materials

Population and data collection

FAMHES is a population-based project, which was mainly performed to investigate environmental and genetic factors, as well as their interrelations. 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. Then, 3593 participants responded for further interviews (response rate = 83.5%). [13] No distinct differences were detected between the men who participated in the interviews and those who did not. All participants signed a form indicating that they had provided their informed consent to study participation. This study was approved by the Medical Ethics Committee of Guangxi Medical University.

All participants were asked to provide blood samples between 8:00 am and 11:00 am, after fasting for at least 8 h (overnight). Then, 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. Serum B12 and FA were detected with electrochemiluminescence immunoassays, while serum HCY was measured with enzymatic cycling methods.

Then, all the participants were invited to complete a comprehensive questionnaire. This process was performed by the trained investigators using a standardized protocol with a face-to-face interview. Essential information (e.g., age, sex, smoking, and drinking) was collected, and complete physical examinations (e.g., height, weight, waistline, and hipline) were performed. Smoking status and alcohol consumption were defined as Yes or No. The marital status was classified into living together (married or cohabitation without marriage) and alone (bachelors or bachelorhood). Meanwhile, according to the years of education, three groups could be defined (0-6 years: Primary education; 7-12 years: Intermediate education; and ≥ 13 years: Superior education). In the physical examination, body weight with thin clothing and height without shoes were measured. Then, body mass index (BMI) was calculated with the formula of weight/(height)^2. The waist circumference was measured at the midpoint between the inferior costal margin and the superior iliac crest in the midaxillary line. The hipline was defined as the maximum circumference over the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference/hipline. 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. The flow of participants' collection is shown in **Figure S1**.

Patient and Public Involvement

Patients and the public were not involved in the development of the research question and design or recruitment of this study.

ED definition and grouping

In this study, the International Index of Erectile Function (IIEF-5) was applied to define ED. [14] The IIEF-5 system has five questions, which mainly cover the conditions of erection confidence, erection firmness, maintenance ability, maintenance frequency, and satisfaction, with the scores ranging from 5 to 25. Each question has six selections. According to the orders of answers, the scores are defined as 0–5. Then, participants can be divided into ED (IIEF-5 ≤ 21) and Non-ED (IIEF-5 > 21) groups. According to the symptoms, ED can also be classified into five groups: none (IIEF-5 score 22-25); mild (17-21); moderate (12-16); and severe symptoms (5-11). [13, 15] In addition, HCY level can also be divided into normal (HCY 5-15μmol/L) and hyperhomocysteinemia (HCY > 15μmol/L). [16]

Participants screening

In order to acquire the eligible participants for this study, we developed rigorous exclusion criteria: (i) incomplete data for the individual information and IIEF-5 score; (ii) incomplete data for HCY, B12, and FA or refused to provide the blood samples; (iii) with diseases such as cardiovascular diseases, inflammatory/immune diseases, and kinds of cancers, which might influence the levels of HCY, B12, and FA (**Table S1**); and (iv) currently taking drugs that might affect the HCY, B12, and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine, or glucocorticoids (**Table S1**). Then, 1381 participants were included for further analyses. The flow for screening the eligible participants is shown in **Figure S2**.

Statistical analysis

Before analysis, HCY, B12, and FA levels were tested for Gaussian distribution with the Shapiro-Wilks test. If data were not Gaussian in distribution, they were logarithmically transformed, in order to ensure the approximate Gaussian distribution. Based on the 22 IIEF-5 scores, two groups were defined (ED and Non-ED), and Student's t-test and the chi-squared (X^2) test were applied in the baseline analysis. Then, linear and logistic regression analyses were used, with the IIEF-5 scores and binary variable (ED or Non-ED) as the dependent factors, respectively. Three adjusted models were used: Unadjusted, Age-adjusted, and Multivariate adjusted. In the Multivariate adjusted model, the covariates were as follows: age, smoking status, alcohol consumption, BMI, and WHR. Among them, BMI and WHR are the indexes applied to estimate obesity. However, BMI tends to evaluate body fatness but has a weak ability to differentiate fatness as central or visceral.[17] 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.[17, 18] Additionally, the predictive effects of BMI and WHR in diseases are different.[19, 20] So, in our study, these two obesity indexes were treated as the co-variates.

Then, the multinomial logistic regression analysis was also performed to discover the potential association between HCY, B12, FA, and ED, along with the order of severity of ED or the changes in the HCY, B12, and FA levels quartile (Q1 < 25%, 25% ≤ Q2 ≤ 50%, 50% < Q3 ≤ 75%, and Q4 > 75%). Additionally, considering the non-negligible influences of age on the risk of ED, we also grouped the participants on the basis of age (< 40, 40-49, 50-59, and ≥ 60 years old). The Bernoulli correction was applied, with the significant threshold of $P < 0.0125$ (= 0.05/4 tests) for multinomial logistic regression analysis. Additionally, according to the groups of marital status and educational status, the logistic regression analyses were also conducted. In these analyses, the missing data was deleted. All statistical tests were two-tailed, which were performed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The threshold for significance was $P < 0.05$.

Results

In the baseline analysis, based on IIEF-5, the ED and Non-ED groups were defined. In line with previous studies, the age of the ED group (37.99 ± 10.75 years) was older than the Non-ED group (34.18 ± 8.47 years, $P < 0.001$). Meanwhile, B12 levels were significantly higher in the ED group ($P = 0.015$). Although, no significant difference was shown for HCY levels, the proportion of hyperhomocysteinemia was higher in the ED group (43.02%) than that in the Non-ED group (37.52%, $P = 0.037$). In addition, the proportion of alcohol consumption ($P = 0.032$) and educational status ($P < 0.001$) were also identified to have statistically significant difference in the two groups (**Table 1**).

Signal for the association between HCY and ED

While we discovered no significant association between HCY levels and ED in the comprehensive analyses (**Table 2-5**), a slight association of HCY with ED was observed in the participants grouped by age, especially in the old men (age ≥ 60) (**Table S2**). Similar relevance was confirmed in the marital status (alone, Unadjusted severe ED: OR = 4.385, 95% CI = 1.070-17.974, P = 0.040; Age-adjusted severe ED: OR = 5.085, 95% CI = 1.195-21.636, P = 0.028) (**Table S3**).

In the latter analysis, the HCY was divided into normal (HCY 5-15 μmol/L) and hyperhomocysteinemia (HCY > 15 μmol/L). The significant association between HCY and ED seemed to be more prominent in the men living alone (Age-adjusted severe ED: OR = 2.448, 95% CI = 1.046-5.733, P = 0.039) (**Table S3**).

B12 level is significantly associated with ED

To investigate the association between ED and B12, we applied linear and logistic regression analyses, resulting in no significant association for B12 in the linear regression analysis (in which IIEF-5 scores were treated as the dependent factor). For the binary logistic regression (the status of ED evaluated by IIEF-5 was treated as the dependent factor), B12 was identified to be associated with ED in the unadjusted model (OR = 1.438, 95% CI = 1.070-1.933, P = 0.016). However, the association signal diminished in other adjusted models (**Table 3**). We next investigated the relationship between B12 and ED, based on the severity grades of ED. Interestingly, the positive correlation between B12 and ED was further confirmed, especially among men 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**). Subsequently, the levels of B12 were divided into quartiles. The result showed that B12 might be significantly associated with 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; and P for trend < 0.001) (**Table 5**).

After adjusting age for the above analyses, the significant association between B12 and ED diminished (**Table 2 and 3** and **Table 5**), suggesting that age cannot be excluded while investigating the relationship between B12 and ED. We thus grouped the participants into four age groups (ages < 40, 40-49, 50-59, and ≥ 60 years old). Our results showed that the significant correlations between B12 and mild ED (IIEF-5 = 17–21) mainly presented in the 40–49 years old age group (OR = 2.907, 95% CI = 1.402-6.026, P = 0.004) (**Table S2**).

Our baseline analysis discovered different proportions of educational status in the ED and Non-ED groups. In order to discuss the influences of marital and educational status in the relevance to ED and B12, we further performed between-group analyses. Similar to previous results, B12 was also identified to be associated with mild ED, even after multivariate adjustment (marital status, living together: OR = 1.501, 95% CI = 1.035-2.175, P = 0.032; alone: OR = 3.449, 95% CI = 1.113-10.692, P = 0.032; and educational status, Intermediate: OR = 1.858, 95% CI = 1.214-2.845, P = 0.004) (**Table S3**).

Discussion

ED is a common disorder, affecting a large number of males. [1–4] Recent studies suggest HCY may be an independent risk factor for ED. [8, 9] In order to test this association, we conducted current study based on the larger population-based FAMHES project. We confirmed that HCY is significantly associated with ED, especially severe ED. Moreover, B12 may also be relevant to mild ED. In contrast, we observed no significant association between FA and ED in our study.

HCY was reported to be associated with many diseases and health conditions, such as psychological disorders, [21, 22] lipid profiles, [23] renal Impairment, [24] and inflammatory/immune factors. [25] Moreover, HCY is also identified to be a useful marker for CVD. [26, 27] Meanwhile, ED

could be a potentially predictive factor for cardiovascular and other chronic diseases. [28] Based on the relevance, it was assumed that HCY might be a risk factor for ED. [8, 9] In consistent with this, we revealed that HCY was significantly associated with ED, especially severe ED. The main mechanism might be that HCY could influence endothelial dysfunction and nitric oxide (NO) diffusion. Previously, in vitro and in vivo studies discovered that HCY could be a toxin for the vasculature by inducing endothelial dysfunction. [29] Additionally, NO is mainly involved in vascular dilation, smooth muscle relaxation (including genitourinary smooth muscle), and permitting penile erection. [30, 31] Studies showed that increased HCY could inhibit NO synthase, thereby probably influencing the production of NO, and the development of ED. [32] So, on the basis of these relevance, we could understand the risk effect of HCY on ED. Additionally, the status of living alone for men would also influence this association, hinting the pathogenesis of psychological factors for ED.

B12 is also known as cobalamin. Similar to FA, it is an important cofactor in methionine synthesis and homocysteine metabolism. [33] Although previous studies identified that FA might be a potential protective factor for ED, [34] no significant association has been detected. In contrast to B12, HCY has been found to protect against ED. [35] Our study also identifies the potential association between B12 and ED, though 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 prominent for mild ED at the higher B12 levels. There are two possible explanations. First, our results suggest that the function of B12 in ED might be dose-dependent. Excessive 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, defense mechanisms are triggered. As a potential protective factor, the absorption of B12 is enhanced. Combining the limited reports, 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.

Limitations

Our study verified the previous conclusions that HCY could increase the risk of ED. However, some limitations still need to be noted: (i) this study is a cross-sectional analysis, which just reflects the status of specific time points and populations; (ii) there are limited numbers of participants with primary educational status. So, the results need to be examined further; (iii) although we have identified a significant association between B12 and ED, the exact mechanisms and effects were unclear until now; and (iv) after multiple testing, no positive association can be detected, suggesting that our results might be unstable. So, further studies will be needed.

Conclusions

ED is one of the most common male diseases. This study was conducted in order to discover the functions of HCY, B12, and FA in ED. Our results confirmed the positive correlations of HCY and ED. Meanwhile, B12 was also likely to be significantly associated with ED. Further studies with larger cohorts of participants should be focused on the potential mechanisms and therapeutic effects of B12 on ED.

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Conflict of Interest

There are no conflicts of interests.

Author Contributions

Y.C., J.L., Z.N.M., and J.W.C. participated in participants' collection, field investigation, design, writing and modification of all the paper. Y.C. and J.L. took part in the statistical analysis. Z.N.M. and J.W.C. provided important advices for this paper. T.Y.L., J.X.L., J.L.L. and G.H.W. provide efforts in the processes of modification.

Data Sharing Statement

The data for this study was available in the supplementary materials. Further questions could be sent to ZN.M (zengnanmo@hotmail.com) and JW.C (chengjiwen1977@foxmail.com).

References

1. Braun M, Wassmer G, Klotz T, et al. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res 2000;12:305-11. [PubMed: 11416833]

2. Pinnock CB, Stapleton AM, Marshall VR. Erectile dysfunction in the community: a prevalence study. Med J Aust 1999;171:353-7. [PubMed: 10590723]

3. Nicolosi A, Moreira ED Jr, Shirai M, et al. Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectile dysfunction. Urology 2003;61:201-6. [PubMed: 10590723]

4. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 1999;84:50-56. [PubMed: 10444124]

5. Kloner RA, Speakman M. Erectile dysfunction and atherosclerosis. Curr Atheroscler Rep 2002;4:397-401. [PubMed: 12162940]

6. Sullivan ME, Keoghane SR, Miller MA. Vascular risk factors and erectile dysfunction. BJU Int 2001;87:838-45. [PubMed: 11412223]

7. Nehra A, Jackson G, Miner M, et al. Diagnosis and treatment of erectile dysfunction for reduction of cardiovascular risk. J Urol 2013;189:2031-8. [PubMed: 23313195]

8. 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. [PubMed: 10510122]

9. 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. [PubMed: 27709655]
10. Long Y, Nie J. Homocysteine in Renal Injury. Kidney Dis (Basel) 2016; 2:80-7. [PubMed: 27536696]
11. Lai WK, Kan MY. Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1-12. [PubMed: 26201664]
12. Sansone M, Sansone A, Romano M, et al. Folate: a possible role in erectile dysfunction? Aging Male. 2017 Nov 20:1-5. [PubMed: 29157083]
13. 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. [PubMed: 25343742]
14. Rosen RC, Cappelleri JC, Smith MD, et al. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res 1999;11:319-26. [PubMed: 10637462]
15. Kupelian V, Araujo AB, Chiu GR, et al. Relative contributions of modifiable risk factors to erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. Prev Med 2010;50:19-25. [PubMed: 19944117]
16. Guo H, Chi J, Xing Y, et al. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res 2009; 129:279-84. [PubMed: 19491420]
17. McDonnold M, Mele LM, Myatt L, et al. Waist-to-Hip Ratio versus Body Mass Index as Predictor of Obesity-Related Pregnancy Outcomes. Am J Perinatol. 2016;33:618-624. [PubMed: 26788786]
18. Suchanek P, Kralova Lesna I, et al. Which index best correlates with body fat mass: BAI, BMI, waist or WHR? Neuro Endocrinol Lett. 2012;33 Suppl 2:78-82. [PubMed: 23183515]
19. Tang B, Han CT, Zhang GM, et al. 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;7:43551. [PubMed: 28272469]
20. Welborn TA, Dhaliwal SS. Preferred clinical measures of central obesity for predicting mortality. Eur J Clin Nutr. 2007;61:1373-9. [PubMed: 17299478]
21. Salagre E, Vizuete AF, Leite M, et al. Homocysteine as a peripheral biomarker in bipolar disorder: A meta-analysis. Eur Psychiatry. 2017;43:81-91. [PubMed: 28371745]
22. Elstgeest LE, Brouwer IA, Penninx BW, et al. Vitamin B12, homocysteine and depressive symptoms: a longitudinal study among older adults. Eur J Clin Nutr 2017;71:468-75. [PubMed: 28145420]
23. Momin M, Jia J, Fan F, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis 2017; 16:54. [PubMed: 28288621]
24. Chen J, Li G, Xu Z, et al. Elevated Plasma Homocysteine Level Increased the Risk of Early Renal Impairment in Acute Ischemic Stroke Patients. Cell Mol Neurobiol 2017;37:1399-1405. [PubMed: 28275883]
25. Li T, Chen Y, Li J, et al. Serum Homocysteine Concentration Is Significantly Associated with Inflammatory/Immune Factors. PLoS One 2015; 10:e0138099. [PubMed: 26367537]
26. Sahu A, Gupta T, Kavishwar A, et al. Cardiovascular Diseases Risk Prediction by Homocysteine in Comparison to other Markers: A Study from Madhya Pradesh. J Assoc Physicians India 2015;63:37-40. [PubMed: 27608690]

27. Yeh JK, Chen CC, Hsieh MJ, et al. Impact of Homocysteine Level on Long-term Cardiovascular Outcomes in Patients after Coronary Artery Stenting. J Atheroscler Thromb 2017;24:696-705. [PubMed: 27803490]

28. Baumann F, Hehli D, Makaloski V, et al. Erectile dysfunction - overview from a cardiovascular perspective. Vasa 2017; 1-7. [PubMed: 28486869]

29. McDowell IF, Lang D. Homocysteine and endothelial dysfuncton: A link with cardiovascular disease. J Nutr 2000;130: 369S-372S. [PubMed: 10721909]

30. Rajfer J, Aronson WJ, Bush PA, et al. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Engl J Med 1992;326:90-94. [PubMed: 1309211]

31. Deanfield J, Donald A, Ferri C, et al. Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension: Endothelial function and dysfunction. Part I: methodological issues for assessment in the different vascular beds: a statement by the working group on endothelin and endothelial factors of the European society of hypertension. J Hypertens 2005;23:7-17. [PubMed: 15643116]

32. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-375. [PubMed: 10475890]

33. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2:299-316. [PubMed: 22254022]

34. Yan WJ, Yu N, Yin TL, et al. A new potential risk factor in patients with erectile dysfunction and premature ejaculation: folate deficiency. Asian J Androl 2014;16:902-906. [PubMed: 25080932]

35. Giovannone R, Busetto GM, Antonini G, et al. Hyperhomocysteinemia as an Early Predictor of Erectile Dysfunction: International Index of Erectile Function (IIEF) and Penile Doppler Ultrasound Correlation With Plasma Levels of Homocysteine. Medicine (Baltimore) 2015;94:e1556. [PubMed: 26426624]

	ED	Non-ED	P
No.	688	693	
Age, years	**37.99±10.75**	**34.18±8.47**	**<0.001[b]**
BMI, Kg/m^2	23.27±3.26	23.37±3.48	0.591[b]

WHR	0.88±0.06	0.88±0.06	0.253[b]
HCY, μmol/L	14.97±4.11	15.34±11.09	0.524[b]
Normal HCY	392 (56.98%)	433 (62.48%)	
Hyperhomocysteinemia	**296 (43.02%)**	**260 (37.52%)**	**0.037[b]**
B12, pg/ml	**718.53±234.37**	**688.74±229.68**	**0.015[b]**
FA, ng/ml	9.56±2.72	9.89±11.28	0.594[b]
Smoke			
Yes	392 (56.98%)	385 (55.56%)	
No	296 (43.02%)	308 (44.44%)	0.594[c]
Drink			
Yes	**586 (85.17%)**	**617 (89.03%)**	
No	**102 (14.83%)**	**76 (10.97%)**	**0.032[c]**
Marital status[e]			
Live together	595 (86.48%)	578 (83.41%)	
Alone	93 (13.52%)	115 (16.59%)	0.110[c]
educational status[a]			
Primary	**19 (2.76%)**	**5 (0.72%)**	
Intermediate	**488 (70.93%)**	**411 (59.39%)**	
Superior	**181 (26.31%)**	**276 (39.89%)**	**<0.001[c]**

Table 1. The characteristics of the eligible participants in the analysis

a. One participant without the information of educational status in the Non-ED group

b. Student's t-test

c. chi-square test

e. The marital status was classified into live together (married or cohabitation without marriage) and alone (bachelors or bachelorhood).

* Normal HCY: 5-15μmol/L; hyperhomocysteinemia: >15umol/L

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

	Unadjusted			Age-adjusted			Multivariate adjusted		
	BETA	95%CI	P	BETA	95%CI	P	BETA	95%CI	P
IIEF-5									
HCY	-0.202	-1.080, 0.676	0.651	0.139	-0.732, 1.009	0.755	0.084	-0.787, 0.956	0.850
Binary HCY	-0.338	-0.817, 0.142	0.167	-0.146	-0.622, 0.330	0.548	-0.186	-0.663, 0.291	0.444
B12	0.048	-0.600, 0.696	0.885	0.212	-0.428, 0.852	0.515	0.404	-0.259, 1.068	0.232
FA	0.112	-0.668, 0.891	0.779	0.388	-0.384, 1.160	0.986	0.324	-0.496, 1.145	0.438

Table 2. The linear regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* IIEF-5 scores were the dependent factor for the linear regression analysis.

	Unadjusted			Age-adjusted			Multivariate adjusted		
Binary	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY	1.137	0.766-1.687	0.524	0.959	0.641-1.435	0.839	0.986	0.658-1.479	0.986
Binary HCY	**1.258**	**1.014-1.560**	**0.037**	1.151	0.923-1.435	0.212	1.174	0.940-1.466	0.157
B12	**1.438**	**1.070-1.933**	**0.016**	1.338	0.992-1.805	0.057	1.311	0.961-1.788	0.087
FA	1.100	0.775-1.561	0.594	0.956	0.668-1.367	0.804	1.041	0.710-1.527	0.835

Table 3. The binary regression analyses for the ED and HCY, B12 and FOL

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* In the binary regression analysis, the ED status (ED: IIEF-5≤21; Non-ED: IIEF-5>21) was treated as the dependent factor.

	HCY			Binary_HCY			B12			FA		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
ED-Unadjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	1.081	0.695-1.681	0.730	1.246	0.980-1.583	0.072	**1.694**	**1.207-2.376**	**0.002**	1.180	0.801-1.740	0.402
Moderate (12–16)	1.258	0.649-2.439	0.497	1.298	0.896-1.880	0.168	1.187	0.715-1.972	0.508	0.960	0.519-1.776	0.896
Severe (5–11)	1.259	0.562-2.820	0.575	1.258	0.799-1.980	0.322	0.891	0.496-1.599	0.698	0.923	0.434-1.963	0.834
ED-age-adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.948	0.607-1.480	0.814	1.166	0.914-1.486	0.217	**1.596**	**1.135-2.244**	**0.007**	1.052	0.710-1.558	0.800
Moderate (12–16)	0.929	0.455-1.898	0.840	1.095	0.747-1.605	0.643	1.409	0.635-1.733	0.851	0.762	0.404-1.437	0.401
Severe (5–11)	1.068	0.467-2.443	0.876	1.150	0.726-1.820	0.552	0.839	0.471-1.495	0.551	0.794	0.370-1.705	0.554
ED- Multivariate adjusted												
None (IIEF-5= 22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.973	0.621-1.524	0.904	1.188	0.929-1.517	0.169	**1.620**	**1.141-2.300**	**0.007**	1.184	0.775-1.808	0.435
Moderate (12–16)	0.965	0.472-1.971	0.922	1.119	0.762-1.643	0.567	0.972	0.576-1.640	0.915	0.777	0.401-1.507	0.456
Severe (5–11)	1.132	0.491-2.613	0.771	1.187	0.748-1.885	0.467	0.717	0.388-1.324	0.288	0.821	0.368-1.831	0.631

Table 4. Multinomial logistic regression for the association between ED and HCY, B12 and FA

* The categorical dependent variables were the various ED groups, based on the IIEF-5. The symptoms of ED were divided into None (IIEF-5= 22-25), Mild (17-21), Moderate (12-16) and Severe (5-11). And the None group (22-25) was treated as the reference.

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

	Unadjusted			Age-adjusted			Multivariate adjusted		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
HCY									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.130	0.838-1.525	0.422	1.022	0.753-1.387	0.887	1.012	0.744-1.377	0.938
Q3	1.292	0.958-1.743	0.094	1.187	0.875-1.610	0.271	1.210	0.890-1.646	0.224
Q4	1.276	0.946-1.720	0.110	1.091	0.803-1.483	0.578	1.103	0.810-1.502	0.534
B12									
Q1	1	1	1	1	1	1	1	1	1
Q2	0.917	0.680-1.236	0.569	0.893	0.659-1.209	0.464	0.899	0.662-1.221	0.496
Q3	0.988	0.733-1.333	0.939	0.972	0.717-1.316	0.853	0.986	0.726-1.338	0.927
Q4	**1.452**	**1.076-1.961**	**0.015**	1.299	0.955-1.765	0.095	1.286	0.945-1.752	0.110
FA									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.313	0.974-1.770	0.074	1.325	0.978-1.795	0.069	1.332	0.981-1.811	0.067
Q3	1.300	0.963-1.755	0.086	1.243	0.916-1.687	0.163	1.286	0.944-1.751	0.111
Q4	1.198	0.888-1.616	0.236	1.094	0.806-1.487	0.564	1.116	0.819-1.522	0.487

Table 5. Association between HCY, B12, FA and ED along with the increased levels of these indexes

* In the Multinomial logistic regression, the levels of HCY, B12, and FA was divided into quartile (Q1<levels of 25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%), 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”.

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

Excluded diseases	Excluded drugs
Hypertension	Vitamins: Vitamins B12, Vitamins C, Vitamins B2, etc.
Diabetes	Antidiabetic medicines: insulin, metformin, Ningestedglinide, etc.
Angina	Non-steroidal anti-inflammatory drugs: aspirin, acetaminophen, indomethacin, diclofenac, celecoxib, etc.
Myocardial infarction	Antibiotics: penicillin, cephalosporins, aztreonam, ofloxacin, clarithromycin, etc.
Stroke	Cimetidine:
Gout	Glucocorticoids: hydrocortisone, prednisone, methylprednisolone, hexadecadrol, etc.
Rheumatoid arthritis	
Prostatitis	
Hepatitis B	
Various cancers	
Parotiditis	
Urolithiasis	
Pelvic floor surgery	

Table S1. The list of diseases and drugs excluded in this study

		No. ED	No. Non-ED	HCY			Binary HCY			B12			FA		
				BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P	BETA/OR	95%CI	P
Ages <40															
IIEF-5		409	531	-0.246	-1.32, 0.837	0.656	-0.269	-0.845, 0.307	0.359	0.627	-0.202, 1.456	0.138	-0.137	-1.135, 0.862	0.788
ED				0.987	0.594-1.639	0.960	1.147	0.876-1.501	0.319	1.207	0.818-1.780	0.344	1.146	0.718-1.829	0.567
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.961	0.547-1.688	0.889	1.185	0.881-1.594	0.263	1.460	0.945-2.255	0.088	1.162	0.692-1.950	0.571
	Moderate			0.794	0.282-2.238	0.662	0.843	0.486-1.462	0.544	0.957	0.444-2.066	0.912	1.015	0.401-2.568	0.974
	Severe			1.429	0.469-4.357	0.530	1.351	0.732-2.495	0.336	0.544	0.223-1.326	0.180	1.276	0.432-3.766	0.659
40-49		176	131												
IIEF-5				0.501	-1.025, 2.207	0.519	-0.109	-1.060, 0.841	0.821	0.320	-0.904, 1.544	0.607	0.990	-0.622, 2.602	0.228
ED				0.842	0.398,-1.778	0.651	1.129	0.710-1.795	0.608	1.692	0.925-3.094	0.088	0.878	0.398-1.937	0.747
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.820	0.358-1.878	0.638	1.135	0.685-1.882	0.623	**2.907**	**1.402-6.026**	**0.004**	1.174	0.495-2.785	0.716
	Moderate			0.951	0.288-3.146	0.935	1.267	0.598-2.682	0.537	0.550	0.224-1.355	0.194	0.380	0.102-1.407	0.147
	Severe			0.845	0.140-5.123	0.855	0.905	0.318-2.578	0.852	0.910	0.243-3.403	0.889	0.579	0.105-3.183	0.530
50-59		69	21												
IIEF-5				4.297	-0.514, 9.108	0.079	1.597	-0.408, 3.602	0.117	-2.046	-4.862, 0.770	0.152	-1.649	-5.387, 2.088	0.383
ED				0.314	0.028-3.584	0.351	0.867	0.309-2.430	0.786	1.521	0.355-6.516	0.572	1.773	0.265-11.867	0.555
	None			1	1	1	1	1	1	1	1	1	1	1	1
	Mild			0.379	0.024-5.901	0.489	0.927	0.299-2.875	0.895	0.859	0.171-4.308	0.853	1.601	0.198-12.945	0.659
	Moderate			0.669	0.030-14.774	0.799	1.326	0.356-4.931	0.674	4.335	0.603-31.187	0.145	1.042	0.086-12.647	0.974
	Severe			0.046	0.001-1.941	0.107	0.358	0.077-1.660	0.189	2.060	0.256-16.573	0.497	3.751	0.263-53.516	0.330
≥60		34	10												
IIEF-5				-0.479	-7.400, 6.442	0.889	-0.116	-3.491, 3.259	0.945	-0.526	-4.308, 3.255	0.779	2.899	-2.726, 8.523	0.303
ED				29.170	0.379-2.243E3	0.128	2.544	0.409-15.822	0.317	0.714	0.089-5.723	0.751	0.523	0.018-15.079	0.705
	None			1	1	1	1	1	1	1	1	1	1	1	1

	Mild	**767.519**	**1.649-3.573E5**	**0.034**	4.093	0.337-49.760	0.269	0.677	0.038-12.139	0.791	0.645	0.008-52.675	0.845
	Moderate	11.236	0.098-1.289E3	0.317	2.266	0.283-18.119	0.441	1.067	0.092-12.408	0.959	2.717	0.049-150.689	0.626
	Severe	70.920	0.291-1.729E4	0.129	3.281	0.316-34.092	0.320	0.424	0.037-4.866	0.491	0.027	0.000-3.577	0.148

Table S2. Association between ED and HCY, B12, FA level in four ages groups (<40, 40-49, 50-59 and ≥60 years) after adjusting multivariate

* Linear regression for the IIEF-5; binary regression analysis for ED; multinomial logistic regression for the severity of symptom of ED

* ED is divided into four groups according to the IIEF-5 scores: None=22-25 scores, Mild=17-21 scores, Moderate=12-16 scores and Severe=5-11 scores. None ED is treated as the reference.

* ED: erectile dysfunction; HCY= homocysteine; B12= vitamin B12; FA= folic acid; BMI: body mass index; WHR: waist hip rate; OR: odd ratio; 95%CI: 95% confidence interval

* Binary_HCY: Normal HCY (5-15μmol/L); hyperhomocysteinemia (>15umol/L)

* Multi-adjusted: age, BMI, WHR, smoke and drink

	ED grading	HCY			Binary_HCY			B12			FA		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Unadjusted													
Marital status													
Live together		1.070	0.698-1.640	0.757	1.237	0.979-1.562	0.074	**1.400**	**1.010-1.940**	**0.044**	1.283	0.860-1.916	0.222
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.077	0.673-1.722	0.758	1.239	0.959-1.601	0.101	**1.484**	**1.033-2.132**	**0.033**	1.295	0.833-2.012	0.251
	Moderate	1.267	0.629-2.555	0.508	1.367	0.920-2.032	0.122	1.105	0.636-1.921	0.723	1.158	0.583-2.303	0.675
	Severe	0.678	0.227-2.027	0.487	0.987	0.566-1.722	0.964	1.534	0.697-3.375	0.288	1.492	0.580-3.841	0.407
Alone		1.523	0.540-4.299	0.426	1.346	0.765-2.370	0.303	1.458	0.705-3.013	0.309	0.577	0.246-1.356	0.207
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.813	0.207-3.194	0.767	1.169	0.580-2.355	0.662	**3.950**	**1.331-11.719**	**0.013**	0.750	0.285-1.976	0.561
	Moderate	0.985	0.128-7.560	0.988	0.812	0.267-2.470	0.714	1.568	0.352-6.975	0.555	0.330	0.058-1.878	0.211
	Severe	**4.385**	**1.070-17.974**	**0.040**	2.249	0.974-5.193	0.058	0.605	0.252-1.450	0.260	0.460	0.116-1.824	0.269
educational status													
Primary		1.586	0.018-142.652	0.841	0.875	0.116-6.579	0.897	5.169	0.188-142.118	0.331	0.399	0.095-1.676	0.209
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	3.257	0.018-596.353	0.657	0.500	0.045-5.514	0.571	4.007	0.136-117.975	0.421	0.455	0.088-2.364	0.349
	Moderate	0.489	0.002-114.662	0.797	1.500	0.136-16.542	0.741	**1.266E^{3}**	**2.303-6.962E^{5}**	**0.026**	0.423	0.060-2.975	0.387
	Severe	2.199	0.007-686.953	0.788	1.000	0.080-12.557	1.000	2.627	0.200-34.591	0.463	0.205	0.009-4.472	0.314
Intermediate		1.667	0.989-2.811	0.055	1.305	0.993-1.716	0.056	**1.513**	**1.049-2.182**	**0.027**	1.187	0.747-1.887	0.469
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.571	0.883-2.795	0.124	1.295	0.958-1.752	0.093	**1.838**	**1.215-2.781**	**0.004**	1.304	0.779-2.181	0.312
	Moderate	1.619	0.692-3.785	0.266	1.265	0.806-1.986	0.307	1.132	0.620-2.067	0.687	0.888	0.411-1.919	0.763
	Severe	2.355	0.876-6.326	0.089	1.429	0.829-2.463	0.199	0.934	0.452-1.926	0.852	1.160	0.451-2.980	0.759
Superior		0.874	0.449-1.699	0.691	1.436	0.986-2.093	0.060	0.938	0.537-1.640	0.823	1.117	0.586-2.129	0.738
	None	1	1	1	1	1	1	1	1	1	1	1	1

	Mild	0.765	0.351-1.669	0.501	1.403	0.924-2.130	0.112	1.156	0.612-2.183	0.655	1.202	0.587-2.462	0.615
	Moderate	1.581	0.567-4.414	0.381	1.800	0.849-3.818	0.126	0.640	0.225-1.821	0.403	1.244	0.345-4.481	0.739
	Severe	0.479	0.064-3.567	0.472	1.170	0.461-2.970	0.741	0.491	0.142-1.691	0.259	0.569	0.116-2.788	0.487
Age-Adjusted													
Marital status													
Live together		9.868	0.559-1.347	0.528	1.110	0.873-1.412	0.394	1.333	0.954-1.861	0.092	1.122	0.743-1.694	0.584
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.940	0.586-1.508	0.796	1.157	0.892-1.501	0.271	1.436	0.996-2.072	0.053	1.176	0.752-1.839	0.478
	Moderate	0.874	0.402-1.902	0.735	1.110	0.735-1.676	0.620	1.015	0.583-1.766	0.958	0.947	0.469-1.909	0.878
	Severe	0.378	0.114-1.258	0.113	0.762	0.428-1.357	0.356	1.353	0.622-2.943	0.445	1.193	0.459-3.097	0.717
Alone		1.611	0.566-4.586	0.371	1.406	0.793-2.493	0.243	1.391	0.653-2.961	0.392	0.622	0.257-1.506	0.292
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.216-3.352	0.817	1.210	0.598-2.451	0.596	**3.742**	**1.258-11.129**	**0.018**	0.799	0.294-2.169	0.659
	Moderate	1.019	0.132-7.836	0.986	0.831	0.272-2.542	0.745	1.492	0.339-6.562	0.597	0.341	0.060-1.947	0.226
	Severe	**5.085**	**1.195-21.636**	**0.028**	**2.448**	**1.046-5.733**	**0.039**	0.450	0.175-1.159	0.098	0.516	0.122-2.174	0.367
educational status													
Primary		0.285	0.001-61.236	0.647	0.212	0.010-4.431	0.317	10.044	0.027-3.674E^{3}	0.444	0.421	0.080-2.228	0.309
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.588	0.004-601.127	0.879	0.228	0.010-5.326	0.357	4.021	0.062-259.697	0.513	0.485	0.093-2.542	0.392
	Moderate	0.001	7.773E^{-8}-6.096	0.116	0.139	0.003-6.581	0.316	3.874E^{3}	1.164-1.289E^{7}	0.046	0.426	0.024-7.587	0.561
	Severe	0.035	1.430E^{-5}-84.089	0.398	0.118	0.002-6.145	0.290	8.466	0.008-8.474E^{3}	0.545	0.162	0.005-5.514	0.311
Intermediate		1.392	0.820-2.365	0.221	1.209	0.915-1.597	0.182	1.384	0.954-2.009	0.087	1.073	0.669-1.722	0.769
	None	1	1	1	1	1		1	1	1	1	1	1
	Mild	1.382	0.774-2.468	0.275	1.229	0.906-1.668	0.186	**1.723**	**1.133-2.620**	**0.011**	1.206	0.717-2.030	0.480
	Moderate	1.145	0.470-2.787	0.766	1.084	0.682-1.724	0.733	0.966	0.529-1.764	0.911	0.754	0.346-1.643	0.478
	Severe	1.915	0.698-5.257	0.207	1.298	0.748-2.254	0.354	0.840	0.409-1.726	0.635	1.022	0.397-2.632	0.964
Superior		0.779	0.391-1.552	0.477	1.324	0.900-1.948	0.154	0.953	0.540-1.684	0.869	0.898	0.461-1.750	0.751

	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.672	0.297-1.521	0.340	1.289	0.841-1.976	0.245	1.175	0.618-2.238	0.623	0.960	0.458-2.012	0.914
	Moderate	1.421	0.486-4.155	0.521	1.583	0.736-3.405	0.240	0.682	0.241-1.926	0.470	0.917	0.244-3.440	0.898
	Severe	0.521	0.074-3.647	0.511	1.202	0.472-3.061	0.699	0.473	0.132-1.690	0.249	0.622	0.125-3.096	0.562
Multivariate adjusted													
Marital status													
Live together		0.894	0.575-1.390	0.619	1.137	0.893-1.448	0.299	1.349	0.962-1.891	0.083	1.176	0.773-1.789	0.449
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.963	0.598-1.548	0.875	1.181	0.909-1.534	0.213	**1.501**	**1.035-2.175**	**0.032**	1.245	0.790-1.961	0.345
	Moderate	0.913	0.420-1.985	0.818	1.136	0.751-1.719	0.547	0.971	0.554-1.701	0.918	0.933	0.455-1.915	0.851
	Severe	0.417	0.126-1.378	0.152	0.812	0.454-1.453	0.483	1.227	0.562-2.675	0.608	1.317	0.494-3.514	0.582
Alone		1.602	0.546-4.698	0.390	1.399	0.779-2.512	0.261	1.288	0.558-2.975	0.553	0.678	0.253-1.820	0.441
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.850	0.206-3.498	0.821	1.199	0.579-2.481	0.625	**3.449**	**1.113-10.692**	**0.032**	0.959	0.279-3.292	0.947
	Moderate	1.130	0.135-9.481	0.910	0.856	0.276-2.654	0.788	1.244	0.261-5.929	0.784	0.336	0.054-2.069	0.239
	Severe	4.181	0.951-19.374	0.058	2.356	0.989-5.616	0.053	0.302	0.084-1.083	0.066	0.598	0.139-2.570	0.490
educational status													
Primary		0.045	0.000-51.900	0.388	0.098	0.002-5.651	0.262	6.248	0.006-6.418E3	0.605	2.823	0.032-247.907	0.649
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.198	$4.915E^{-5}$-794.766	0.702	0.224	0.004-13.29	0.473	0.001	3.247E-10-3.819E3	0.376	1.806	0.007-479.698	0.836
	Moderate	$2.184E^{-12}$	$2.988E^{-28}$-$1.597E^{4}$	0.150	0.004	1.429E-6-8.838	0.157	**NA**	**NA**	**0.043**	3.396	$0.006\text{-}1.820E^{3}$	0.703
	Severe	1.972E-9	$1.318E^{-24}$-$2.952E^{6}$	0.261	0.008	$4.142E^{-6}$-15.343	0.210	**$8.065E^{-217}$**	**0.000-0.203**	**0.049**	0.516	0.001-457.324	0.849
Intermediate		1.431	0.842-2.432	0.186	1.223	0.925-1.618	0.158	1.428	0.981-2.081	0.063	1.092	0.677-1.764	0.717
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	1.401	0.782-2.510	0.257	1.238	0.910-1.682	0.174	**1.858**	**1.214-2.845**	**0.004**	1.268	0.748-2.149	0.378
	Moderate	1.225	0.502-2.990	0.656	1.105	0.694-1.761	0.673	0.954	0.519-1.755	0.880	0.707	0.319-1.568	0.394
	Severe	2.044	0.744-5.615	0.165	1.350	0.776-2.351	0.288	0.754	0.364-1.562	0.448	0.969	0.367-2.562	0.950

Superior		0.812	0.405-1.627	0.557	1.360	0.920-2.011	0.123	0.977	0.550-1.738	0.938	0.889	0.449-1.762	0.737
	None	1	1	1	1	1	1	1	1	1	1	1	1
	Mild	0.702	0.308-1.598	0.399	1.325	0.859-2.044	0.204	1.202	0.632-2.287	0.575	0.898	0.419-1.926	0.783
	Moderate	1.437	0.483-4.270	0.514	1.556	0.718-3.370	0.262	0.647	0.217-1.930	0.435	1.056	0.274-4.063	0.937
	Severe	0.598	0.092-3.890	0.591	1.333	0.513-3.459	0.555	0.496	0.123-2.002	0.325	0.715	0.145-3.529	0.681

Table S3. The logistic regression analyses (binary and multinomial) for the association between ED and HCY, B12 and FA in the subgroups of marital status and educational status

* In the educational status, the Primary group only contains 24 participants. So, some results of regression analyses were exaggerated with these limited data

* Multivariate adjusted: age, BMI, WHR, smoke and drink

* HCY= homocysteine; B12= vitamin B12; FA= folic acid

* Binary_HCY: Normal HCY (5-15µmol/L); hyperhomocysteinemia (>15umol/L)

* ED status: none (IIEF-5 score 22–25); mild (17–21); moderate (12–16); and severe (5–11).

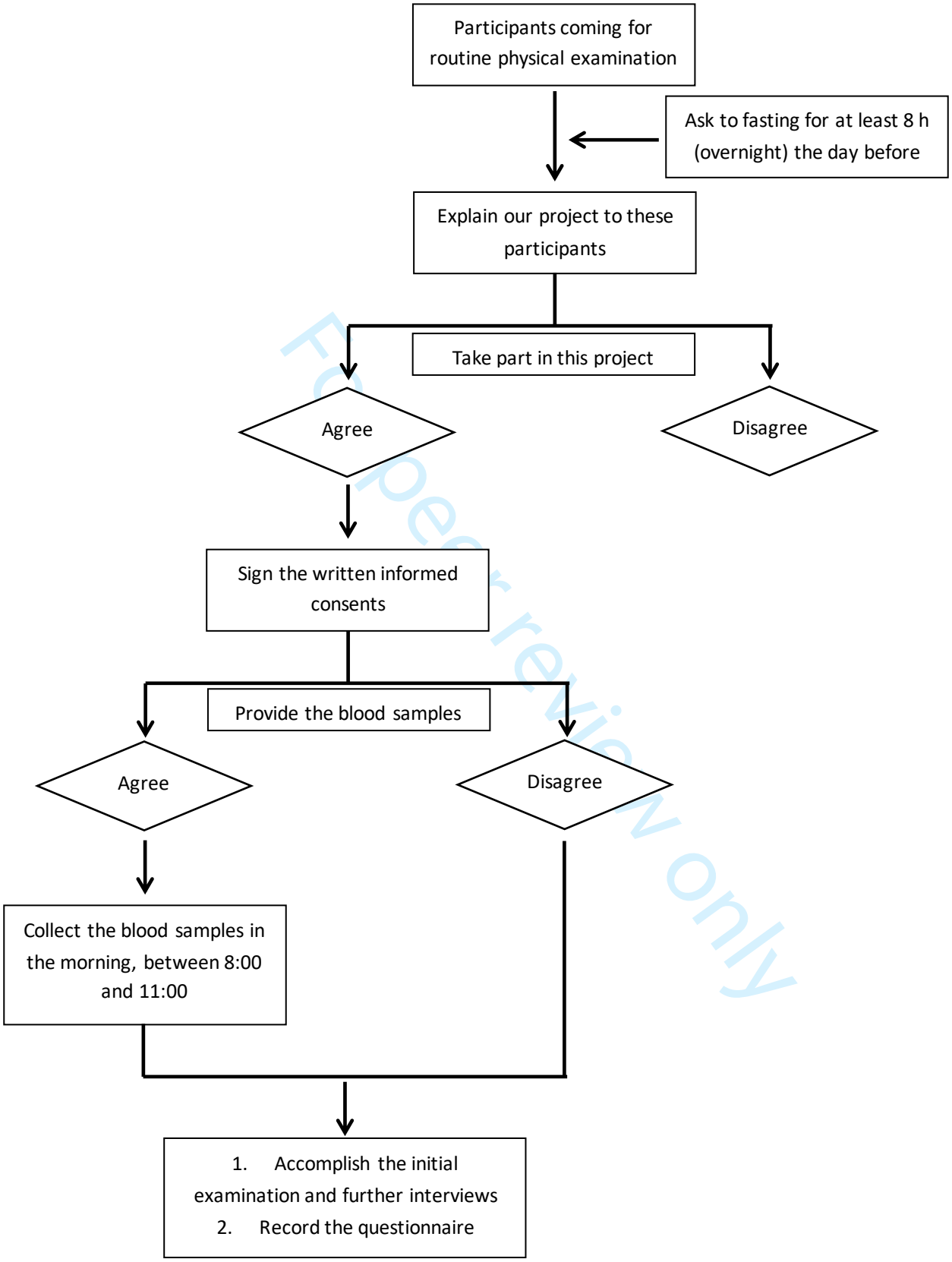

Figure S1. The flow of participants' collection

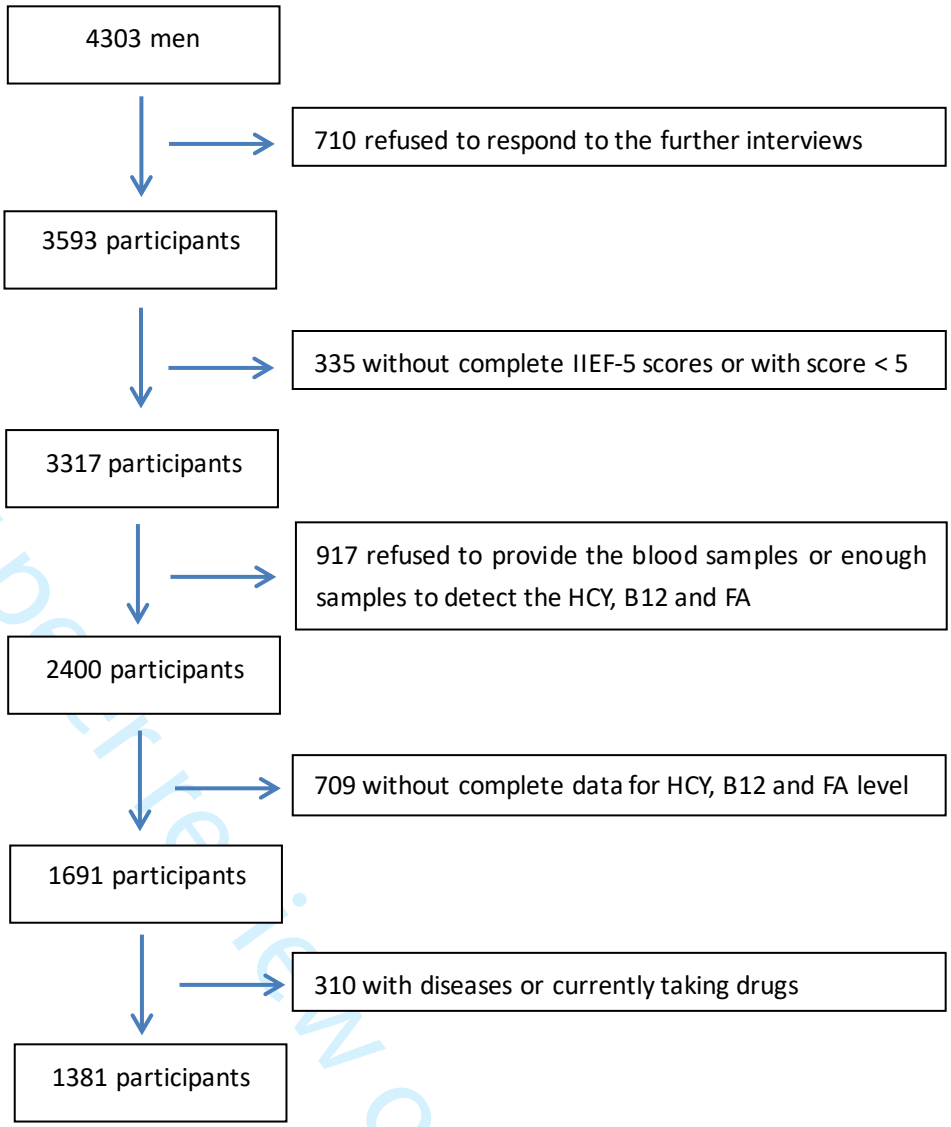

Figure S2. The flow for screening the eligible participants

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	*4*
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6
		(*b*) Report category boundaries when continuous variables were categorized	5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.