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Association between hyperhomocysteinaemia and the risk of all-cause and cause-specific mortality among adults in the USA

Wenyan Zhao[1], Yan Lin[1], Huibo He[1], Honglei Ma[1], Wei Yang[1], Qian Hu[1], Xi Chen[1]*† and Faliang Gao[2,3]*†

[1]*Center for General Practice Medicine, Department of General Practice Medicine, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, Zhejiang, People's Republic of China*
[2]*Center for Rehabilitation Medicine, Department of Neurosurgery, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, Zhejiang, People's Republic of China*
[3]*Key Laboratory of Endocrine Gland Diseases of Zhejiang Province, Hangzhou, People's Republic of China*

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Abstract

Hyperhomocysteinaemia (HHcy) is associated with all-cause mortality in some disease states. However, the correlation between HHcy and the risk of mortality in the general population has rarely been researched. We aimed to evaluate the association between HHcy and all-cause and cause-specific mortality among adults in the USA. This study analysed data from the National Health and Nutrition Examination Survey database (1999–2002 survey cycle). A multivariable Cox regression model was built to evaluate the correlation between HHcy and all-cause and cause-specific mortality. Smooth curve fitting was used to analyse their dose-dependent relationship. A total of 8442 adults aged 18–70 years were included in this study. After a median follow-up period of 14·7 years, 1007 (11·9 %) deaths occurred including 197 CVD-related deaths, 255 cancer-related deaths and fifty-eight respiratory disease deaths. The participants with HHcy had a 93 % increased risk of all-cause mortality (hazard ratio (HR) 1·93; 95 % CI (1·48, 2·51)), 160 % increased risk of CVD mortality (HR 2·60; 95 % CI (1·52, 4·45)) and 82 % increased risk of cancer mortality (HR 1·82; 95 % CI (1·03, 3·21)) compared with those without HHcy. For unmeasured confounding, E-value analysis proved to be robust. In conclusion, HHcy was associated with high risk of all-cause and cause-specific (CVD, cancer) mortality among adults aged below 70 years.

Keywords: **Hyperhomocysteinaemia: Mortality: Adults: National Health and Nutrition Examination Survey**

Homocysteine (Hcy), a sulphur-containing amino acid, is a metabolite of methionine[1]. Over the past 10 years, Hcy has been described as a well-established risk factor for the development of arteriosclerotic vascular diseases[2]. At present, the generally accepted reference range for Hcy level is 5–15 μmol/l. Hcy concentrations greater than 15 μmol/l are considered indicative of hyperhomocysteinaemia (HHcy), which occurs in 5–7 % of the general population[3,4]. The association between total plasma homocysteine (tHcy) and the risk of some disease outcomes has been studied. A growing body of evidence indicates that elevated tHcy is associated with an increased risk of CVD[2,5,6,7,8]. After adjustments were made for known cardiovascular risk factors, one meta-analysis of twelve prospective studies suggested that a 25 % reduction in Hcy levels can reduce the risk of ischaemic heart disease by 11 % and the risk of stroke by 19 %[9]. Another recent meta-analysis showed that participants with higher Hcy levels had a 58 % increased risk of stroke and 55 % increased risk of ischaemic stroke, compared with those with lower Hcy levels[10]. In addition, elevated tHcy levels are related to several age-related diseases, such as essential hypertension[11], Parkinson's disease[12], Alzheimer's disease[13], diabetes[14] and osteoporosis[15].

The relationship between tHcy levels and the risk of all-cause mortality has also been analysed. Multiple studies have indicated that HHcy is associated with a higher risk of mortality in populations with specific chronic diseases, such as coronary artery disease[16], type 2 diabetes[17] and renal failure[18], as well as in renal transplant recipients[19] and frail individuals[20]. However, it should be noted that these populations already have a higher overall risk of mortality due to their underlying disease conditions. Furthermore, large-scale epidemiological data on the relationship between tHcy levels and the risk of all-cause mortality are still lacking, especially in the general population.

Considering the results of previous research, some pertinent questions remain unanswered. First, does a linear or non-linear

Abbreviations: CAD, coronary atherosclerotic heart disease; Hcy, homocysteine; HHcy, hyperhomocysteinaemia; HR, hazard ratio; SBP, systolic blood pressure.

* **Corresponding author:** Dr F. Gao, email gaofaliang1985@126.com; X. Chen, email vipxichen2021@126.com

† These authors contributed equally to this work and share correspondence.

relationship exist between tHcy and the risk of mortality? Second, is the relationship between tHcy and mortality based on the independent role of tHcy or on a product of important confounding factors such as vitamin B_{12}, folate, renal function or disease susceptibilities? Thus, we conducted this study to investigate the association between HHcy and the risk of all-cause and cause-specific mortality in a large, population-based sample of adults in the USA.

Methods

Study design

In this study, we analysed data extracted from the National Health and Nutrition Examination Survey (NHANES, 1999–2002), a complex, stratified, multistage probability survey conducted by the Centers for Disease Control and Prevention (CDC). The NHANES programme began in the early 1960s and collected data on the demographics, lifestyle and health status of the US population using questionnaires. Biomarker data of the participants were also collected. This annual cross-sectional survey examines a nationally representative sample of about 5000 persons from counties across the country. Since NHANES 1999–2000, data for public use have been released almost every 2 years. We pooled data from two 2-year survey cycles of NHANES (1999–2000 and 2001–2002) for this study. We conducted a prospective secondary analysis by linking NHANES data with mortality data from the National Death Index. The National Center for Health Statistics (NCHS) Ethics Review Board approved the NHANES programme and released its documents for public use. Written informed consent was obtained from each participant. More information regarding the methodological details of the NHANES is available on the NHANES official website (www.cdc.gov/nchs/nhanes/). We obtained the NHANES datasets from DataDryad (https://doi.org/10.5061/dryad.d5h62).

Study population

We conducted a secondary analysis of data from two 2-year NHANES survey cycles: 1999–2000 and 2001–2002. Given the possibility of survival bias among old adults, the participants were limited to adults aged 18–70 years (*n* 9446). Individuals without data on tHcy levels (*n* 996) and all-cause mortality (*n* 8) were excluded. Finally, 8442 eligible participants were enrolled in the final analysis (Fig. 1).

Exposure variable and endpoint

The exposure variable was tHcy level (μmol/l) and HHcy. HHcy was defined as a tHcy level greater than or equal to 15 μmol/l. Hcy was measured using the Abbott IMX analyser in 1999–2000, the Abbott Homocysteine IMX in 2001 and the Abbott Axsym in 2002. The details of the tHcy measurement process are available at http://cdc.gov/nchs/nhanes.

The primary endpoint was all-cause mortality, and the secondary endpoint was cause-specific mortality including CVD, cancer and respiratory disease mortality. The mortality information including cause and time of death was obtained from the

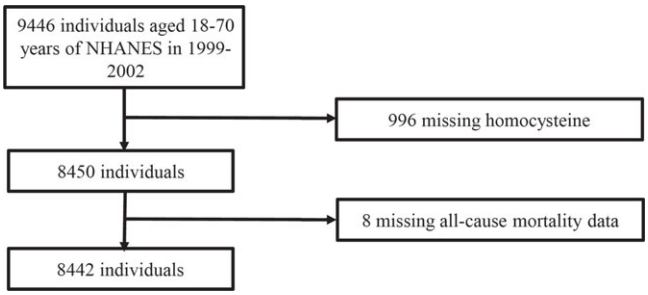

Fig. 1. Flow chart of participants.

2015 NCHS Public-Use Linked Mortality Files. Follow-up data were taken for the period from the date of participation in the NHANES survey until the date of death or 31 December 2015. Cause of mortality was ascertained by the NCHS based on the International Classification of Diseases, 10th revision. CVD mortality was defined as death due to diseases of the heart (Codes: I00–I09, I11, I13, I20–I51) and cerebrovascular disease (Codes: I60–I69). Cancer mortality was defined as death due to malignant neoplasms (Codes: C00–C97), and respiratory disease mortality was defined as death due to chronic lower respiratory diseases (Codes: J40–J47), influenza and pneumonia (Codes: J09–J18).

Covariates

Statistical analyses were adjusted for *a priori* covariates based on well-known risk factors for mortality[21]. The following covariates were included as continuous variables: age, BMI (kg/m^2), mean systolic blood pressure (SBP, mmHg), mean diastolic blood pressure (mmHg), C-reactive protein (mg/dl) level, glycohaemoglobin (%) level, total cholesterol (mg/dl) level, albumin (g/dl) level, alanine aminotransferase (U/l), aspartate aminotransferase (U/l), γ-glutamyl transferase (U/l), alkaline phosphatase (U/l), uric acid (mg/dl) level, blood urea nitrogen (mg/dl), estimated glomerular filtration rate (ml/min per 1·73 m^2), serum vitamin B_{12} (pg/ml) level, serum folate (ng/ml) level, dietary factors (total monounsaturated fatty acids (g), total polyunsaturated fatty acids (g), total saturated fatty acids (g), total fat intake (g), protein intake (g), dietary fibre (g), energy intake (kcal)) and supplement use (vitamin B_{12} (mg), folic acid (mg)). The categorical variables included sex, race (grouped as non-Hispanic White, Black, Mexican American, other Hispanic or other), education status (dichotomised as below high school diploma, high school diploma or any training above high school diploma), smoking status (grouped as never smoker, current smoker, former smoker), alcohol consumption (classified as less than 5 g or more than 5 g drinks/d), physical activity (classified as sedentary, low, moderate and high level based on the distribution of metabolic equivalent of task (MET)-minute levels in the present NHANES sample), history of diseases (coronary atherosclerotic heart disease (CAD), hypertension, diabetes and cancer) and medication use (lipid-lowering drugs, antihypertensive drugs and glucose-lowering drugs). CAD was defined as a self-reported physician diagnosis of CAD. Hypertension was defined as meeting any of the following criteria: SBP ≥ 135 mmHg, diastolic blood pressure ≥ 85 mmHg or self-reported physician diagnosis of hypertension. Diabetes was defined as a self-

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reported physician diagnosis of diabetes or a fasting glucose concentration >126 mg/dl. Cancer was defined as a self-reported cancer or malignancy (any type).

Statistical analyses

Statistical analyses were performed following the guidelines of the CDC (https://wwwn.cdc.gov/nchs/nhanes/tutorials/default.aspx). Each participant in the NHANES survey was assigned a sample weight[22]. The proposed weighting methodology in the analytical guidelines of the NCHS was adopted. Continuous variables were presented as mean values with their standard error using weighted linear regression models, and categorical variables were presented as proportions ± SE using weighted chi-square tests.

A generalised additive model and smooth curve fitting (restricted cubic spline) based on Cox proportional hazards models were applied to estimate the relationship between tHcy level and mortality. We utilised four models simultaneously according to the STROBE guidelines: model 1 (not adjusted for any covariates), model 2 (adjusted for age (smooth), sex, race), model 3 (adjusted for all covariates shown in Table 1 except for dietary factors and supplement use) and model 4 (adjusted for all covariates shown in Table 1). Age covariates were entered into the equation using smooth curve fitting to account for the potential non-linear relationship between age and mortality. Hazard ratios (HR) and 95 % CI were estimated in the four models. tHcy level was included as a continuous variable and categorical variable (with and without HHcy). Cumulative survival rate analysis was performed using Kaplan–Meier curves with log-rank statistics according to different groups (with and without HHcy).

Subgroup analyses were performed according to age group (above and below 50 years), sex, race/ethnicity, survey cycles, lifestyle (smoking status, alcohol consumption and physical activity), history of chronic disease, medication use, BMI, estimated glomerular filtration rate, vitamin B_{12} level (tertile grouping) and folate level (tertile grouping) using stratified Cox proportional hazards models.

To confirm the robustness of our results, we quantified unmeasured confounders between HHcy and all-cause mortality by calculating E-values[23], as unmeasured confounding factors may overturn the observed association between HHcy and all-cause mortality. E-values can estimate the validity required for a confounding factor.

All tests were two-sided and statistical significance was set at $P < 0{\cdot}05$. All analyses were performed using the R statistical software package (http://www.R-project.org, The R Foundation for Statistical Computing), EmpowerStats (http://www.empowerstats.com, X&Y Solution, Inc.) and Free Statistics software versions 1.5[24].

Results

Baseline characteristics of the participants

The weighted distribution of the baseline characteristics of the participants according to the presence or absence of HHcy is shown in Table 1. There were 235 (2·78 %) participants with HHcy. Compared with the participants without HHcy, participants with HHcy were slightly older, to be males and more likely to be drinker and former smokers, have a less physically activity, have a lower education level (below high school diploma), have a diagnosis of CAD, hypertension and/or diabetes, have a significantly higher SBP, C-reactive protein level, aspartate aminotransferase level, γ-glutamyl transferase level, alkaline phosphatase level, uric acid level and blood urea nitrogen level, have a lower estimated glomerular filtration rate, serum vitamin B_{12} and serum folate level and take less total monounsaturated fatty acids, vitamin B_{12} and folic acid.

Endpoints of mortality

A total of 8442 individuals (1999–2000 survey cycle: 3978 subjects; 2001–2002 survey cycle: 4464 subjects) aged 18–70 years with 119 364·8 person-years of follow-up (median follow-up duration, 14·7 years; interquartile range, 13·7–15·8 years) were included in the final data analysis. We noted that a total of 1007 (11·9 %) deaths occurred including 197 CVD-related deaths, 255 cancer-related deaths and fifty-eight respiratory disease deaths during follow-up. The participants with HHcy had a higher all-cause and cause-specific mortality rate (per 1000 person-years) than those without HHcy. *P*-values were all less than 0·001 (Table 2).

Association between total plasma homocysteine and hyperhomocysteinaemia an all-cause and cause-specific mortality

Four models were constructed using a generalised additive model to analyse the effect of tHcy levels and HHcy on all-cause and cause-specific mortality. The HR and 95 % CI for these equations are shown in Table 3. In model 4 (the fully adjusted model), a 1 μmol/l higher tHcy level was associated with 4 % increased risk of all-cause mortality (HR 1·04; 95 % CI (1·03, 1·05)), 6 % increased risk of CVD mortality (HR 1·06; 95 % CI (1·03, 1·09)), 1 % increased risk of cancer mortality (HR 1·01; 95 % CI (0·98, 1·05)) and an 8 % increased risk of respiratory disease mortality (HR 1·08; 95 % CI (1·03, 1·14)). The results of the association between tHcy level and cancer mortality did not reach statistical significance.

Participants with HHcy had a 93 % increased risk of all-cause mortality (HR 1·93; 95 % CI (1·48, 2·51)), a 160 % increased risk of CVD mortality (HR 2·60; 95 % CI (1·52, 4·45)), an 82 % increased risk of cancer mortality (HR 1·82; 95 % CI (1·03, 3·21)) and a 146 % increased risk of respiratory disease mortality (HR 2·46; 95 % CI (0·89, 6·81)) compared with those without HHcy. However, the *P* value was greater than 0·05 (not significant) for the risk of respiratory disease mortality.

Analyses of the dose–response relationship between total plasma homocysteine and all-cause and cause-specific mortality

The association between tHcy level and all-cause and cause-specific mortality was evaluated on a continuous scale using a generalised additive model and smooth curve fitting (restricted cubic spline method) based on Cox proportional hazards models. The

Table 1. Characteristics of study participants
(Mean values and standard errors)

Characteristics	Total (*n** 8442)		Without HHcy (*n* 8207)		With HHcy (*n* 235)		
	Mean/*n* (%)	SE	Mean/*n* (%)	SE	Mean/*n* (%)	SE	*P*
Age, years	41·34	0·29	41·15	0·30	48·6	1·19	<0·001
Male	49·05	0·48	48·7	0·47	62·2	4·04	0·003
Race/ethnicity							
Non-Hispanic White	69·44	1·83	69·5	1·81	67·01	3·73	0·406
Black	10·82	1·22	10·71	1·22	14·85	2·26	0·026
Mexican American	7·77	0·92	7·82	0·93	6·01	1·09	0·093
Other Hispanic	7·18	1·6	7·17	1·59	7·58	3·04	0·863
Other race/ethnicity	4·79	0·69	4·8	0·71	4·54	1·11	0·846
Education							
< High school	20·68	0·79	20·46	0·79	28·63	3·75	0·025
High school	25·43	1·04	25·46	1·06	24·36	4·36	0·808
> High school	53·89	1·45	54·08	1·48	47·02	4·78	0·168
Alcohol consumption, g/d							
<5	83·18	0·7	83·49	0·69	72·24	3·66	0·001
≥5	16·82	0·7	16·51	0·69	27·76	3·66	0·001
Smoking							
Never	50·25	1·36	50·71	1·40	33·57	2·88	<0·001
Current	23·53	0·99	23·42	1·03	27·85	3·11	0·182
Former	26·21	0·96	25·87	0·96	38·58	4·47	0·005
Physical activity							
Sedentary	19·69	1·08	19·4	1·09	30·56	3·46	0·002
Low	27·33	0·94	27·22	0·97	31·17	3·77	0·320
Moderate	19·52	0·69	19·53	0·72	19	3·39	0·885
High	33·46	0·96	33·85	0·96	19·27	3·4	0·002
Co-morbidities							
CAD	4·70	0·34	4·45	0·34	14·13	1·88	<0·001
Hypertension	22·32	0·88	21·75	0·83	43·93	4·6	<0·001
Diabetes	6·70	0·38	6·51	0·36	13·9	2·79	0·001
Cancer	5·79	0·44	5·72	0·46	8·55	2·49	0·216
Medication use							
Statin	5·00	0·36	4·96	0·38	6·66	1·49	0·256
ACEi	3·80	0·32	3·54	0·33	13·60	1·81	<0·001
Antidiabetic drugs	2·73	0·26	2·68	0·26	4·53	1·22	0·054
Physical examination							
BMI, kg/m^2	27·97	0·14	27·96	0·14	28·35	0·51	0·471
Mean systolic, mmHg	117·29	0·35	117·12	0·38	124·49	1·51	0·000
Mean diastolic, mmHg	71·91	0·28	71·85	0·29	74·10	1·17	0·083
Laboratory data							
CRP, mg/dl	0·41	0·01	0·40	0·01	0·61	0·09	0·035
Glycohaemoglobin	5·40	0·02	5·40	0·02	5·48	0·07	0·287
Total cholesterol, mg/dl	197·01	0·79	196·87	0·83	202·47	2·88	0·087
Albumin, g/dl	4·39	0·01	4·39	0·01	4·35	0·04	0·277
ALT, U/l	26·51	0·48	26·11	0·30	41·40	13·45	0·264
AST, U/l	24·44	0·25	24·25	0·23	31·78	2·78	0·011
GGT, U/l	30·35	0·52	29·63	0·45	57·14	6·67	0·000
ALP, U/l	73·29	0·78	73·07	0·80	81·63	1·69	0·000
Uric acid, mg/dl	5·31	0·02	5·28	0·02	6·38	0·18	<0·001
BUN, mg/dl	13·36	0·11	13·28	0·11	16·44	0·87	0·001
eGFR, ml/min per 1·73 m^2	105·33	0·51	105·83	0·52	86·40	3·16	<0·001
Serum vitamin B_{12}, pg/ml	542·02	21·57	545·77	22·04	398·69	28·68	0·000
Serum folate, ng/ml	14·17	0·22	14·27	0·22	10·20	1·06	0·001
Dietary							
TMFA intake, g	31·84	0·26	31·95	0·25	28·05	1·78	0·032
TPFA intake, g	17·34	0·19	17·39	0·18	15·47	1·29	0·145
TSFA intake, g	27·92	0·25	27·99	0·25	25·59	1·24	0·054
Total fat intake, g	84·94	0·66	85·14	0·64	77·19	4·22	0·063
Protein intake, g	84·08	0·68	84·22	0·70	79·11	4·18	0·246
Dietary fibre intake, g	15·95	0·26	16·00	0·26	14·07	0·98	0·053
Energy intake, kcal	2282·68	15·67	2284·02	15·66	2233·11	91·53	0·582
Supplement use							
Vitamin B_{12}, mg	14·43	1·14	14·75	1·16	2·75	1·43	<0·001
Folic acid, mg	123·19	4·57	125·51	4·58	37·20	11·51	<0·001

HHcy, hyperhomocysteinaemia; CAD, coronary atherosclerotic heart disease; ACEi, angiotensin-converting enzyme inhibitors; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; TMFA, total monounsaturated fatty acids; TPFA, total polyunsaturated fatty acids; TSFA, total saturated fatty acids.
* Unweighted number of observations in dataset.
Continuous variables were calculated by weighted linear regression model. Categorical variables were calculated by weighted chi-square test.

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Table 2. The endpoints in participants without and with HHcy

Endpoints	Total	Without HHcy	With HHcy	*P*
All-cause mortality				<0·001
Person-years	119 365	116 671·8	2693	
No. of events	1007	905	102	
Mortality rate (per 1000 person-years)	8·4	7·8	37·9	
CVD mortality				<0·001
Person-years	119 365	116 671·8	2693	
No. of events	197	171	26	
Mortality rate (per 1000 person-years)	1·7	1·5	9·7	
Cancer mortality				<0·001
Person-years	119 365	116 671·8	2693	
No. of events	255	235	20	
Mortality rate (per 1000 person-years)	2·1	2·0	7·4	
Respiratory disease mortality				<0·001
Person-years	119 365	116 671·8	2693	
No. of events	58	52	6	
Mortality rate (per 1000 person-years)	0·5	0·4	2·2	

HHcy, hyperhomocysteinaemia.

fully adjusted smooth curve fitting showed a linear association between tHcy level and all-cause and cause-specific mortality (Fig. 2).

Subgroup analyses

The results of the subgroup analyses of the association between HHcy and all-cause mortality are presented in Fig. 3. The association between HHcy and all-cause mortality in the stratified analysis was consistent with that in the multivariable Cox regression analysis, except for Mexican American.

Survival analyses

Kaplan–Meier analysis showed that the survival probability among participants with HHcy was significantly lower compared with those without HHcy (both $P < 0·0001$) (Fig. 4).

Sensitivity analyses

To test the robustness of the primary results, we calculated an E-value to assess the effect of unmeasured confounding factors. The association between HHcy and the risk of all-cause mortality was found to be robust, unless the HR of all-cause mortality risk of an unmeasured confounder was >3·06.

Discussion

Our findings

In this study, we used the generalised additive model to illustrate the relationship between HHcy and the risk of all-cause and cause-specific mortality among adults under the age of 70 years from the general population of the USA. After adjusting for indicators such as demographics, traditional cardiovascular risk factors and laboratory test results, we observed that HHcy was associated with an increased risk of all-cause and cause-specific mortality after a median follow-up of 14·7 years. However, the risk of respiratory disease mortality did not reach statistical significance.

Previous studies

Our results are consistent with the follow-up report of older Framingham subjects[25] and residents of Jerusalem[26], which states that tHcy was an effective predictor of all-cause mortality. There is increasing evidence that elevated Hcy levels are associated with an increased risk of all-cause mortality[27,28]. A meta-analysis of nineteen studies with 4340 subjects showed that elevated Hcy levels were associated with a 3·19-fold increased risk of all-cause mortality[29]. Another meta-analysis of ten prospective studies with 11 061 participants found that stroke and ischaemic stroke risk increases in a dose-dependent manner with increases in tHcy level[10]. This is consistent with the results of the present study. However, one previous study reported that a high tHcy level is not associated with the risk of cardiovascular mortality after 10·3 years of follow-up in healthy individuals aged 20–59 years[30]. Nonetheless, that study may have been limited by the selection of relatively young healthy subjects. In addition, some important confounding variables, such as folic acid and vitamin B levels, were not adjusted in that study.

Possible explanations for our findings

HHcy can cause atherosclerosis and promote thrombus formation. This mechanism may cause endothelial dysfunction through increased oxidative stress[31,32,33,34]. Hcy can also affect the properties of the extracellular matrix, increase smooth muscle cell proliferation and induce platelet enrichment[35,36,37]. Studies have shown that Hcy level is positively correlated with age[38]. In the present study, participants with HHcy were older and more likely to have complications. Hcy level may be an indirect marker of serious diseases. The association between HHcy and the risk of all-cause and CVD mortality decreased significantly after adjusting for age and sex. Moreover, the subgroup analysis showed that there is a strong correlation between HHcy and the risk of all-cause mortality among participants with risk factors for CVD, such as current smokers, drinkers and those with a history of hypertension and diabetes. And, the participants with HHcy were more likely to be drinker, former smokers, have a less physically activity, have a diagnosis of CAD, hypertension

Table 3. Association of HHcy with the risk of all-cause and cause-specific mortality (Hazards ratios and 95 % confidence intervals)

	Model 1†			Model 2‡			Model 3§			Model 4‖		
Homocysteine	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*
All-cause mortality												
Per 1-μmol/l increment	1·08	1·07, 1·09	<0·001	1·06	1·05, 1·07	<0·001	1·04	1·03, 1·05	<0·001	1·04	1·03, 1·05	<0·001
HHcy												
No	Ref			Ref			Ref			Ref		
Yes	5·10	4·15, 6·26	<0·001	2·99	2·43, 3·68	<0·001	1·85	1·44, 2·39	<0·001	1·93	1·48, 2·51	<0·001
CVD mortality												
Per 1-μmol/l increment	1·09	1·08, 1·10	<0·001	1·07	1·05, 1·09	<0·001	1·06	1·03, 1·09	<0·001	1·06	1·03, 1·09	<0·001
HHcy												
No	Ref			Ref			Ref			Ref		
Yes	6·90	4·57, 10·44	<0·001	3·29	2·16, 5·00	<0·001	2·27	1·34, 3·84	0·002	2·60	1·52, 4·45	0·001
Cancer mortality												
Per 1-μmol/l increment	1·07	1·05, 1·09	<0·001	1·04	1·01, 1·06	0·005	1·02	0·99, 1·05	0·283	1·01	0·98, 1·05	0·410
HHcy												
No	Ref			Ref			Ref			Ref		
Yes	3·82	2·42, 6·03	<0·001	2·29	1·44, 3·63	0·000	1·89	1·10, 3·26	0·021	1·82	1·03, 3·21	0·041
Respiratory disease mortality												
Per 1-μmol/l increment	1·09	1·06, 1·12	<0·001	1·07	1·04, 1·11	<0·001	1·08	1·03, 1·13	0·003	1·08	1·03, 1·14	0·003
HHcy												
No	Ref			Ref			Ref			Ref		
Yes	5·34	2·29, 12·43	<0·001	2·84	1·21, 6·69	0·017	2·49	0·91, 6·76	0·074	2·46	0·89, 6·81	0·083

HR, hazards ratio; HHcy, hyperhomocysteinaemia.

* Cox proportional hazards models were used to estimate HR and 95 % CI.

† Model 1: no covariates were adjusted.

‡ Model 2: adjusted for age (smooth), sex, race/ethnicity.

§ Model 3: adjusted for age (smooth), sex, race/ethnicity, education status, smoking status, alcohol consumption, physical activity, coronary atherosclerotic heart disease, hypertension, diabetes, cancer, glucose-lowering drugs, statin use, ACEi use, BMI, SBP, DBP, CRP, glycohaemoglobin, total cholesterol, albumin, ALT, AST, GGT, ALP, uric acid, BUN, eGFR, serum vitamin B_{12}, serum folate.

‖ Model 4: adjusted for age (smooth), sex, race/ethnicity, education status, smoking status, alcohol consumption, physical activity, coronary atherosclerotic heart disease, hypertension, diabetes, cancer, glucose-lowering drugs, statin use, ACEi use, BMI, SBP, DBP, CRP, glycohaemoglobin, total cholesterol, albumin, ALT, AST, GGT, ALP, uric acid, BUN, eGFR, serum vitamin B_{12}, serum folate, total monounsaturated fatty acids, total polyunsaturated fatty acids, total saturated fatty acids, total fat intake, protein intake, dietary fibre, energy intake, and supplement use (vitamin B_{12}, folic acid).

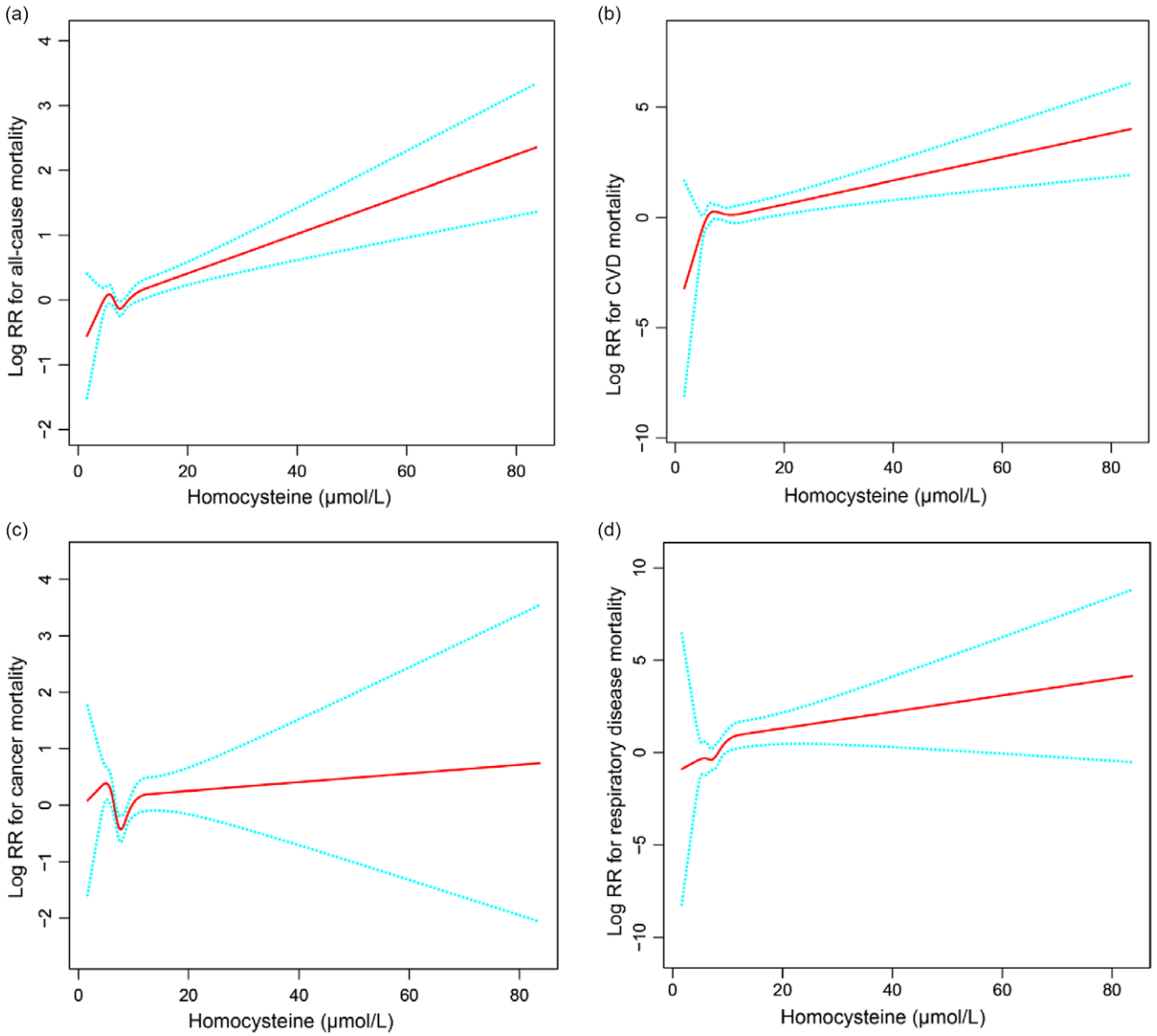

Fig. 2. Dose–response associations of homocysteine level with risk of all-cause (a), CVD (b), cancer (c) and respiratory disease mortality (d). The red solid line represents the estimated risk of all-cause and cause-specific mortality, with cyan dashed lines showing 95 % CI. Analyses were adjusted for age (smooth), sex, race/ethnicity, education status, smoking status, alcohol consumption, physical activity, coronary atherosclerotic heart disease, hypertension, diabetes, cancer, glucose-lowering drugs, statin use, ACEi use, BMI, SBP, DBP, CRP, glycohaemoglobin, total cholesterol, albumin, ALT, AST, GGT, ALP, uric acid, BUN, eGFR, serum vitamin B_{12}, serum folate, total monounsaturated fatty acids, total polyunsaturated fatty acids, total saturated fatty acids, total fat intake, protein intake, dietary fibre, energy intake, and supplement use (vitamin B_{12}, folic acid). ACEi, angiotensin-converting enzyme inhibitor; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

and diabetes. People who smoke heavily for a long time may be prone to vitamin deficiency due to less intake of vegetables and fruits in their diet, which in turn leads to increased tHcy levels. Chronic alcohol consumption affects post-translational modification of hepatic methionine synthase[39]. Inhibition of methionine synthase by alcohol reduces re-methylation of Hcy, resulting in HHcy[40]. It may be a combination of multiple factors such as age, sex, unhealthy lifestyle (smoking and drinking habits, less physically activity) and chronic diseases that may interact with each other affecting the methionine-homocysteine cycle, thereby influencing the final adverse outcomes. Azarpazhooh *et al.*[41] found the metabolic syndrome, smoking and HHcy interact with each other and ultimately contribute to increased cardiovascular risk. Another study also showed that a healthy lifestyle such as physical activity, intaking of more fruit and quitting smoking can help prevent HHcy[42].

Hypertension and hyperhomocysteinaemia

Subgroup analysis in our study showed that HHcy was associated with a higher risk of all-cause mortality in participants with hypertension than those without hypertension, which was in line with our previous study[43]. The Hordaland Homocysteine Study[44] that included 16 176 individuals indicated that tHcy level was positively correlated with SBP and diastolic blood

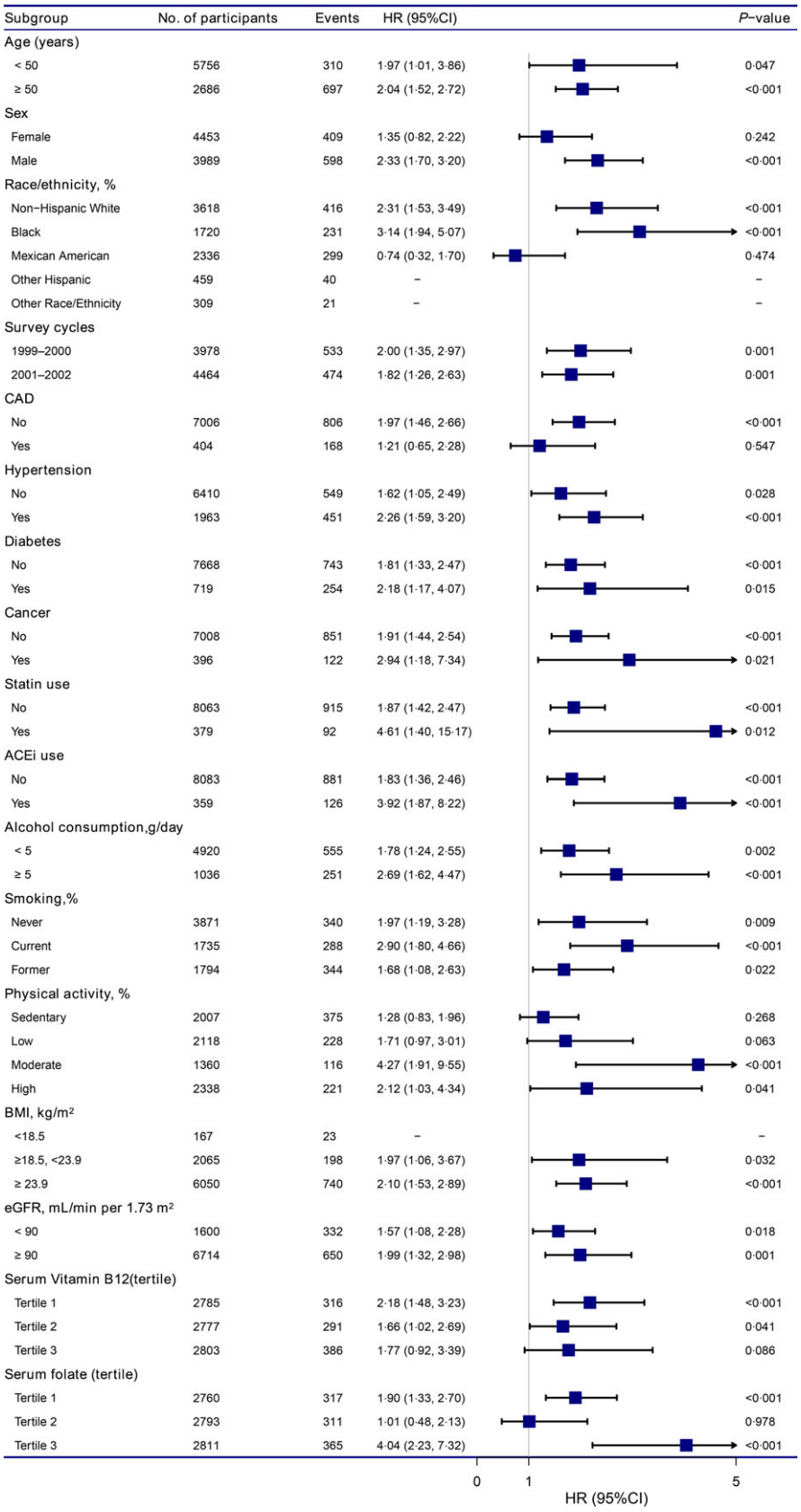

Fig. 3. Association between hyperhomocysteinaemia and all-cause mortality according to subgroup. Analyses were adjusted for age (smooth), sex, race/ethnicity, education status, smoking status, alcohol consumption, physical activity, CAD, hypertension, diabetes, cancer, glucose-lowering drugs, statin use, ACEi use, BMI, SBP, DBP, CRP, glycohaemoglobin, total cholesterol, albumin, ALT, AST, GGT, ALP, uric acid, BUN, eGFR, serum vitamin B_{12}, serum folate, total monounsaturated fatty acids, total polyunsaturated fatty acids, total saturated fatty acids, total fat intake, protein intake, dietary fibre, energy intake, and supplement use (vitamin B_{12}, folic acid), except for the stratification variable. ACEi, angiotensin-converting enzyme inhibitor; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CAD, coronary atherosclerotic heart disease.

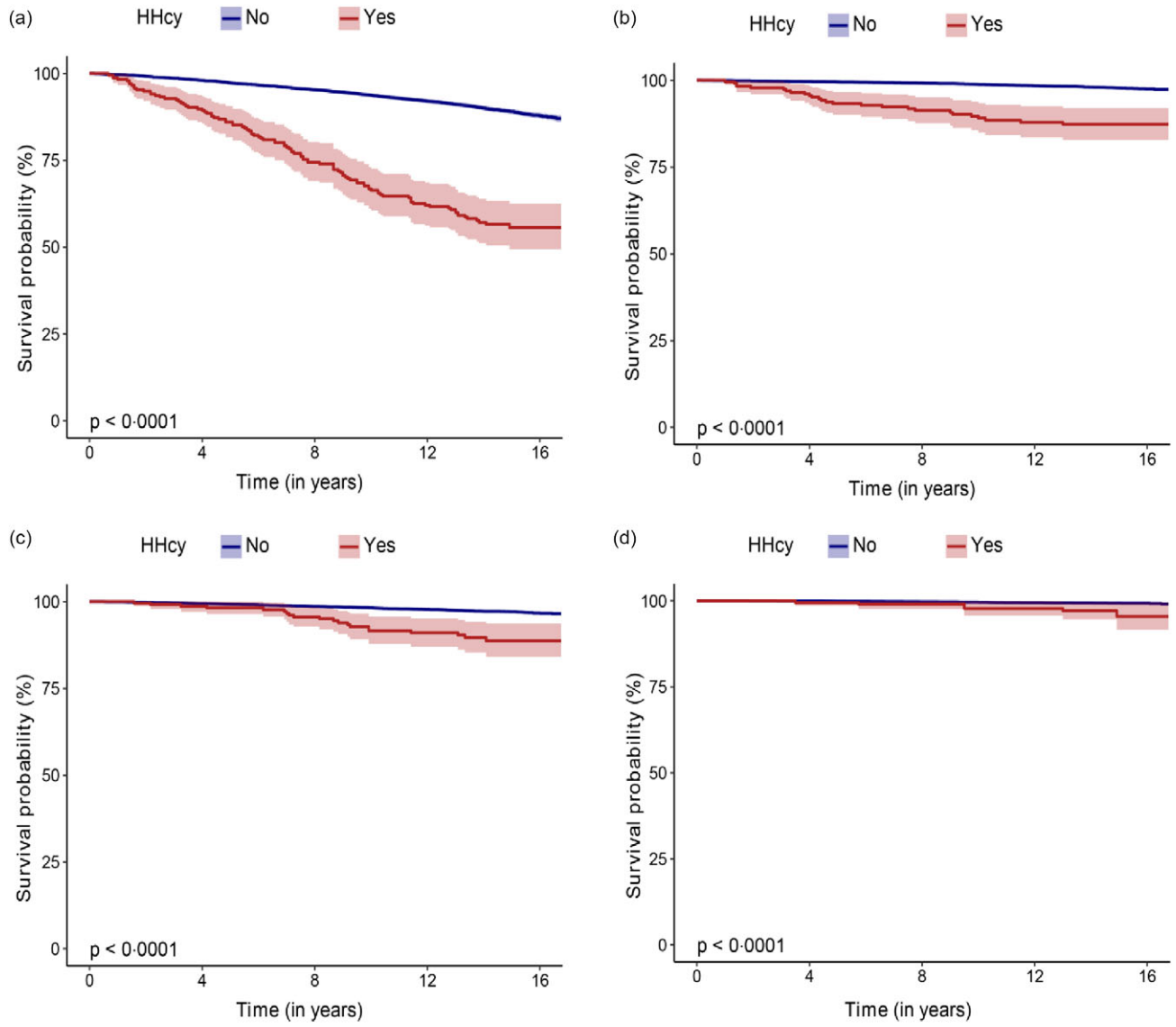

Fig. 4. Kaplan–Meier curves for all-cause (a), CVD (b), cancer (c) and respiratory disease mortality (d). Unadjusted Kaplan–Meier estimates for all-cause and cause-specific mortality for HHcy. HHcy, hyperhomocysteinaemia.

pressure. Results from another NHANES study[45] also suggested that a 5-μmol/l increase in Hcy levels was associated with 0·7 and 0·5 mmHg increases in SBP and diastolic blood pressure, respectively. Zhong *et al.*[11] found that elevated Hcy levels were associated with risk of essential hypertension. This may be due to the destruction of elastic fibres and increased arterial stiffness as a result of Hcy. Symons *et al.*[46] observed that the carotid elasticity was less in hyperhomocysteinemic rats compared with control rats.

Vitamin B_{12}, folate and hyperhomocysteinaemia

Vitamin B_{12} and folate are important factors in Hcy metabolism and important determinants of tHcy concentration. Elevated tHcy levels may reflect a lack of folate and vitamin B_{12}[47,48]. B-complex vitamins can reduce tHcy levels by promoting Hcy metabolism[49]. Bertoia *et al.* revealed a negative correlation between high folate intake and tHcy levels[50]. Studies have shown that two-thirds of patients with HHcy have lower plasma folate and vitamin B_{12} concentrations than normal[51]. A study of 1041 relatively older adults also showed that vitamin B_{12} plays an important role in the pathogenesis of HHcy[38]. Similarly, in the present study, we found that participants with HHcy had lower vitamin B_{12} and folate levels. In addition, our subgroup analysis showed that tHcy levels showed a stronger relationship with the risk of all-cause mortality in the lower tertile of vitamin B_{12} than in the higher tertile, a finding which is consistent with that of previous studies[52]. However, a meta-analysis of a large, randomised trial did not show that vitamin B therapy has a beneficial effect on the mortality of individuals at risk of CVD or those suffering from CVD[53]. Therefore, further observation and clinical trials are necessary to develop appropriate primary prevention strategies.

Effects of drugs and hyperhomocysteinaemia

Numerous epidemiological studies have shown that statin use is significantly associated with a reduction in all-cause and cardiovascular mortality[54,55,56] and angiotensin-converting enzyme inhibitors can significantly reduce the risk of all-cause mortality in patients with co-morbidities such as hypertension, diabetes, CHD and chronic kidney disease[57,58]. However, in our subgroup analysis, participants with HHcy who took statins or angiotensin-converting enzyme inhibitors were associated with a higher risk of all-cause mortality than those who did not. This may be because of a higher prevalence of co-existing cardiovascular risk factors such as hyperlipidaemia and hypertension in participants with HHcy as they had a higher percentage of statin and angiotensin-converting enzyme inhibitor drug usage. In addition, statins may not reduce tHcy concentrations. A meta-analysis of seven studies showed no significant alteration in Hcy levels following treatment with statins[59].

Limitations

The limitations of our study should be noted as well. First, the prevalence of HHcy in kidney transplant recipients is higher than that in the general population[60]. Our study data did not include information on history of kidney transplantation; therefore, we could not assess the role of kidney transplants in the relationship between HHcy and the risk of mortality. Second, the possibility of the residual confounding effect of incomplete adjustment of some cardiovascular risk factors cannot be excluded. However, an E-value analysis was conducted to quantify the potential impact of unmeasured confounders. The results showed that an unknown confounder was unlikely to explain the effect of the risk of all-cause mortality. Third, all blood tests including tHcy levels were based on a single measure, and regression dilution bias may underestimate the strength of the association. Therefore, larger-scale studies among the general population are needed to estimate the strength of the correlation between tHcy level and mortality more accurately.

Conclusion

The main finding of this study was that HHcy was associated with high risk of all-cause and cause-specific (CVD, cancer) mortality among adults aged below 70 years in the USA, which suggests that maintaining tHcy at normal levels may be beneficial in reducing the risk of mortality. Future prospective studies are needed to evaluate the clinical benefit of a Hcy-lowering intervention.

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The authors declare that no conflicts of interest exist.

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