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Prevalence of hyperhomocysteinaemia in a Chinese elderly population

Quan-Gang Qu[1], Jin-Ji Gao[2] and Jian-Meng Liu[1,*]

[1]Institute of Reproductive and Child Health, Peking University Health Science Center, 38 Xueyuan Road, Haidian District, Beijing 100191, People's Republic of China: [2]The Maternal and Child Health Care Hospital, Yuanshi County, Shijiazhuang City, Hebei Province, People's Republic of China

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Abstract

Objective: To evaluate plasma homocysteine (Hcy) levels and prevalence rates of hyperhomocysteinaemia (HHCY) in elderly Chinese individuals.
Design: A cross-sectional study.
Setting: The study was conducted in 2006 in two counties from the north and the south of China.
Subjects: A total of 810 individuals aged 65–74 years were recruited. Demographic characteristics and lifestyle factors were assessed through questionnaire interviews and physical examination. Hcy and folate levels were measured in blood samples. The distribution of Hcy level was analysed according to Hcy-related factors.
Results: Northerners had higher Hcy levels (18·42 μmol/l) than southerners (10·20 μmol/l). Plasma Hcy was higher in men than in women and greater in smokers than in non-smokers. The prevalence rate of HHCY was 51·6 % in the north and 10·1 % in the south ($P < 0·001$). Hcy and plasma folate showed an inverse correlation (Spearman's $r = -0·44$, $P < 0·001$; partial $r = -0·229$, $P < 0·001$). Region, gender, alcohol consumption and plasma folate were associated with HHCY among these elderly populations.
Conclusions: The results demonstrated that plasma Hcy levels and the prevalence rates of HHCY in Chinese elderly are considerably higher than those found in other countries, and substantial regional variations occur within China. The predominant determining factors of HHCY were region, gender, alcohol consumption and plasma folate. The elevated Hcy levels among elderly Chinese populations need to be decreased urgently.

Keywords
Homocysteine
Hyperhomocysteinaemia
Elderly
Chinese

Hyperhomocysteinaemia (HHCY) has been suggested as a risk factor for developing coronary artery disease, myocardial infarction and stroke[1,2]. Epidemiological studies have documented that, in general populations, the most common risk factor for having HHCY is suboptimal or deficient vitamin (e.g. folate) status. Moreover, homocysteine (Hcy) levels also increase significantly with age and HHCY prevalence rates have been reported to be higher in the elderly than in other age groups[3–6]. Thus, risk factors for HHCY may vary by life stage and nutritional background. Findings from younger age groups and populations with better nutritional status are unlikely to be applicable to the elderly. However, in view of the fact that both overall nutritional status and health status are associated with elevated risk of HHCY, the prevalence rates of HHCY among elderly populations should be evaluated.

Developed countries have carried out several large-scale population-based surveys to evaluate Hcy levels in the elderly, but there is a paucity of equivalent data from developing countries. In China, previous studies conducted among elderly individuals were mostly hospital-based, with limitations on study design, selection criteria and sample size. Cross-sectional surveys addressing the issue of differential relationships between Hcy levels and demographic characteristics and lifestyle factors among elderly Chinese populations (aged 65–74 years) are not yet available.

Thus the main purposes of the present study were to characterize the distribution of Hcy levels and identify determining factors associated with HHCY in Chinese individuals aged 65–74 years who live in northern and southern counties of China.

Materials and methods

Study design and population

We chose two counties as field sites: one (Yuanshi County, Hebei Province) in the north (near Beijing) and

*Corresponding author: Email liujm@pku.edu.cn

the other (Nanfeng County, Jiangsu Province) in the south (near Shanghai). In each region, two to four townships were selected so as to provide sufficient potentially eligible volunteers. Study participants were local residents and from the selected townships. An eligible person was aged 65–74 years in 2006. The first stage of the study involved providing local information and distributing informed consent forms to 1600 potentially eligible individuals in the two regions; 1200 (75 %) individuals agreed to participate in the recruitment process. Recruitment included questionnaire interviews, physical examinations and blood sample collection. Trained health workers were responsible for each recruitment step according to standard protocols. Researchers from Peking University were in charge of quality control. On the basis of data collected through recruitment, eligible participants were ascertained by researchers. Those who met any exclusion criterion could not participate in the study. Exclusion criteria included known CHD, hypertension, cancer, severe renal and liver diseases, diabetes, current use (≤6 months) of vitamins or other supplements, and taking medications known to interfere with folate metabolism (e.g. methotrexate). In our study, the main reasons for exclusion were hypertension (60 %), other chronic diseases (22 %) and vitamins taken in the past 6 months (4 %). Eventually, 810 (67·5 %) eligible individuals from 1200 consenting individuals were included in the study, with 405 subjects in each region. Within regions, the participants were distributed equivalently by gender and age groups (65–69 and 70–74 years old). Therefore, our analyses were based on 810 individuals with complete data.

Covariates

Demographic information and lifestyle factors were obtained by questionnaire interviews. BMI was calculated as kg/m^2 from weight and height values. Current smoking was defined as smoking more than 1 cigarette/d for at least 1 month and still smoking. Individuals who were ex-smokers were counted as 'ever smoking' as a dichotomous variable. The rest were considered non-smokers. Alcohol consumption (including liquor, beer and wine) was classified into two categories: non-drinker and drinker.

Blood sample collection and homocysteine analyses

The 810 eligible participants were required to fast overnight before blood collection. Blood samples were drawn and collected in K_3EDTA-containing Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA), held at 4°C and centrifuged at 3000***g*** for 15 min. Plasma and red blood cells were separated and frozen at −20°C within 1 h after collection. All specimens were transported on dry ice to the central laboratory at the Institute of Reproductive and Child Health, Peking University, and stored at −70°C until the assays were performed. Plasma folate concentrations were determined by a microbial assay (*Lactobacillus casei*) using a ninety-six-well plate as described by O'Broin and Kelleher[7]. Plasma Hcy measurements were carried out by HPLC with fluorometric detection. In the laboratory, intra- and inter-assay CV across the full range of concentrations were 9 % for plasma folate and 8 % for Hcy. Repeat Hcy concentrations were measured for a sample of fifty individuals in our laboratory after all assessments were completed, and Spearman's correlation coefficient between the before and after values for the same person was 0·86 ($P < 0·001$). In addition, the assessment of plasma folate was replicated for a sample of 100 individuals by the chemiluminescence method (Beckman Coulter, Fullerton, CA, USA) in a qualified laboratory. The Spearman's correlation coefficient of two values performed by various methods was 0·93 ($P < 0·01$).

Individuals were classified as having HHCY if they had elevated Hcy concentration ≥16·0 μmol/l[8]. Individuals were classified as having plasma folate deficiency if they had plasma folate concentration ≤6·8 nmol/l[9].

Ethical approval

The study was approved by the Institutional Review Boards of Peking University Health Science Center. All invited participants provided oral informed consent.

Statistical methods

Because the distributions of Hcy and plasma folate concentrations were positively skewed, natural logarithmic transformations were used to normalize their distributions. The geometric means as well as the 95 % confidence intervals were also determined. Student's t test and one-way ANOVA were used to compare means. The significance of categorical variables was assessed by the χ^2 test or Fisher's exact test. Logistic regression was used to estimate crude odds ratios of each variable alone in developing HHCY. Adjusted odds ratios were also determined after controlling for other factors (region, age, gender, BMI, occupation, educational background, hypertension, smoking and alcohol consumption). Spearman's correlation coefficients and probabilities were calculated for the relationship between Hcy and plasma folate in continual quantitative variables. Subgroup analyses for certain risk factors were also conducted in females according to menopausal status and in males according to current smoking status.

The data were analysed with the SPSS statistical software package version 11·0 (SPSS, Beijing, China). All P values were two-sided at α level of 0·05.

Results

Selected characteristics of the study population are shown separately by region in Table 1. The demographic characteristics were similar between southerners and northerners. No significant differences were observed in current smoking, health status and medicine use.

Table 1 Characteristics of the study population by region: elderly Chinese individuals (*n* 810) aged 65–74 years, 2006

	North (*n* 405)	South (*n* 405)	*P* value†
Age (years)			0·23
Mean	68	69	
SD	3·32	2·86	
Male (%)	49·9	49·6	0·94
Ethnicity, Han (%)	99·5	99·5	–
Occupation, farmer (%)	95·6	86·1	<0·001
Education, lower than junior school (%)	93·1	95·8	0·06
Chronic disease family history, yes (%)	2·7	17·5	<0·001
Current health status, wellness (%)	97·8	99·3	0·08
Medicine use, yes (%)	16	17	0·71
Current smoking, yes (%)	25·4	27·2	0·58
Ever smoking, yes (%)	26·4	38·0	<0·001
Alcohol consumption, yes (%)	15·9	28·4	<0·001
BMI (kg/m^2)			0·004
Mean	23·12	23·79	
SD	3·32	3·25	
Systolic blood pressure (mmHg)			0·38
Mean	141	140	
SD	16	17	
Diastolic blood pressure (mmHg)			<0·001
Mean	82	74	
SD	8	8	
Hb (g/l)			<0·001
Mean	151	137	
SD	17	17	
Plasma folate (nmol/l)			<0·001
Geometric mean	10·06	14·17	
95 % CI	9·57, 10·57	13·44, 14·94	
Plasma folate deficiency (%)	31·0	7·7	<0·001

†*P* value from *t* test for quantitative variables and χ^2 test for qualitative variables.

Compared with southerners, northerners had lower average BMI, less alcohol consumption, less smoking exposure (as indicated by ever smoking), and fewer had a family history of chronic disease. In addition, northerners were much more likely to have higher Hb levels and diastolic blood pressure than southerners, whereas their systolic blood pressures were similar.

The mean plasma folate levels were relatively low in this study population, but southerners appeared to be in better nutritional status than northerners, with significantly higher levels and corresponding lower deficiency rates.

Homocysteine concentrations

The distribution curves of plasma Hcy were skewed positively, but the curve for northerners had a very long tail towards high values (Fig. 1). Geometric means of Hcy according to demographic and lifestyle factors by region are shown in Table 2. Overall, the mean Hcy level of northerners was 18·42 μmol/l (95 % CI 17·40, 19·50 μmol/l) and nearly twice the level of southerners, i.e. 10·20 μmol/l (95 % CI 9·84, 10·58 μmol/l), thus being significantly higher in the north than in the south ($P<0·01$).

Gender and smoking status differences in Hcy concentrations among both northerners and southerners were notable: men had higher Hcy levels than women, smokers higher than non-smokers. The absolute differences in Hcy concentrations between genders were of approximately similar magnitude as the corresponding absolute difference between smokers and non-smokers, and were greater than those for other factors. In each region, Hcy levels were not significantly influenced by other factors, except educational background in the south ($P<0·01$, Table 2).

Prevalence rate of hyperhomocysteinaemia

The prevalence rate of HHCY was significantly higher in the north than in the south ($P<0·01$), as shown in Table 3. Overall, HHCY (Hcy ≥ 16·0 μmol/l) was detected in 209 (51·6 %) northerners and forty-one (10·1 %) southerners, i.e. nearly five times as many in the north as in the south.

In each region, the prevalence rates of HHCY were observed to be higher in men than women and in smokers than non-smokers. However, the gender and smoking status differences were found to be significant only in the north ($P<0·01$ for both), while neither of them was of significance in the south ($P=0·13$). No statistically significant differences in HHCY prevalence rates were observed for other factors (Table 3).

We applied a logistic regression model to determine the association between each factor and HHCY, as shown in Table 4. Results showed that northerners had nine-fold higher odds of having HHCY than those living in the south. Men had a nearly 75 % increased risk of HHCY compared with women. In addition, those who were

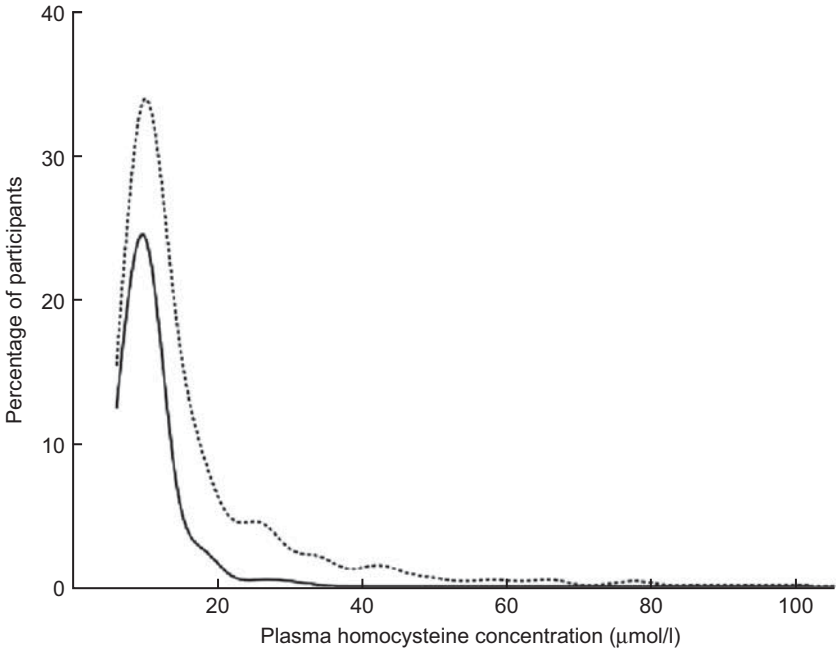

Fig. 1 Distribution of plasma homocysteine concentrations among elderly Chinese individuals aged 65–74 years in the south (*n* 405; ——) and the north (*n* 405; - - - -) of China, 2006

Table 2 Plasma homocysteine levels (μmol/l) according to demographic and lifestyle factors by region: elderly Chinese individuals (*n* 810) aged 65–74 years, 2006

	North			South		
	n	Geometric mean	95 % CI	*n*	Geometric mean	95 % CI
Overall	405	18·42	17·40, 19·50	405	10·20	9·84, 10·58
Age						
65–69 years	203	17·68	16·36, 19·11	203	9·99	9·44, 10·57
70–74 years	202	19·18	17·66, 20·91	202	10·42	9·94, 10·92
Gender						
Male	202	21·14**	19·54, 23·00	201	11·14**	10·52, 11·78
Female	203	16·06	14·87, 17·33	204	9·36	8·96, 9·78
Occupation						
Farmer	387	18·34	17·31, 19·46	347	10·20	9·79, 10·62
Others	18	20·04	14·85, 27·04	58	10·24	9·40, 11·15
Educational background						
Illiterate	175	17·91	16·44, 19·51	204	9·57**	9·14, 10·02
Junior school or higher	230	18·81	17·43, 20·35	201	10·89	10·29, 11·52
BMI (kg/m^2)						
<24	247	18·24	17·02, 19·58	224	10·43	9·90, 10·99
≥24	158	18·70	16·95, 20·63	181	9·93	9·44, 10·44
Hypertension						
Yes	270	18·89	17·58, 20·32	199	10·06	9·57, 10·57
No	135	17·51	15·97, 19·22	206	10·35	9·80, 10·92
Current smoking						
Yes	103	21·03**	19·03, 23·50	110	10·98**	10·15, 11·87
No	302	17·60	16·45, 18·83	295	9·93	9·53, 10·34
Ever smoking						
Yes	107	21·31**	19·29, 23·80	154	10·99**	10·31, 11·70
No	298	17·48	16·34, 18·69	251	9·75	9·33, 10·19
Alcohol consumption						
Yes	64	18·13	15·74, 20·88	115	10·35	9·79, 10·95
No	339	18·49	17·36, 19·69	290	10·15	9·69, 10·63

**$P < 0·01$.

farmers, with higher education and those with hypertension were associated with a greater risk of HHCY to some extent. Interestingly, alcohol consumption showed protective effects for alcohol drinkers, with about 40 % (unadjusted OR = 0·58) or 50 % (adjusted OR = 0·50) reduced risk for elevated Hcy compared with non-drinkers.

Table 3 Hyperhomocysteinaemia prevalence rates (%) according to demographic and lifestyle factors by region: elderly Chinese individuals (*n* 810) aged 65–74 years, 2006

	North (*n* 405)			South (*n* 405)		
	n	Mean	95 % CI	*n*	Mean	95 % CI
Overall	209	51·6	46·6, 56·6	41	10·1	7·4, 13·6
Age						
65–69 years	98	48·3	41·3, 55·4	18	8·9	5·5, 13·9
70–74 years	111	55·0	47·8, 61·9	23	11·4	7·5, 16·8
Gender						
Male	123	60·9**	53·8, 67·6	25	12·4	8·4, 18·0
Female	86	42·4	35·5, 49·5	16	7·8	4·7, 12·7
Occupation						
Farmer	200	51·7	46·6, 56·8	36	10·4	7·5, 14·2
Others	9	50·0	26·8, 73·2	5	8·6	3·2, 19·7
Educational background						
Illiterate	85	48·6	41·0, 56·2	14	6·9	3·9, 11·5
Junior school or higher	124	53·9	47·2, 60·5	27	13·4	9·2, 19·1
BMI (kg/m^2)						
<24	128	51·8	45·4, 58·2	25	11·2	7·5, 16·2
≥24	81	51·3	43·2, 59·3	16	8·8	5·3, 14·2
Hypertension						
Yes	140	51·9	45·7, 57·9	19	9·6	6·0, 14·7
No	69	51·1	42·4, 59·8	22	10·7	7·0, 15·9
Current smoking						
Yes	62	60·2*	50·1, 69·6	12	10·9	6·0, 18·6
No	147	48·7	42·9, 54·5	29	9·8	6·8, 14·0
Ever smoking						
Yes	65	60·8*	50·8, 69·9	18	11·7	7·3, 18·1
No	144	48·3	42·5, 54·2	23	9·2	6·0, 13·6
Alcohol consumption						
Yes	31	48·4	35·9, 61·2	9	7·8	3·9, 14·7
No	177	52·2	46·8, 57·6	32	11·0	7·8, 15·4

*$P<0·05$, **$P<0·01$.

Table 4 Crude and adjusted odds ratios, and their 95 % confidence intervals, of hyperhomocysteinaemia by demographic and lifestyle factors: elderly Chinese individuals (*n* 810) aged 65–74 years, 2006

	Hyperhomocysteinaemia					
Factor	Yes, *n*	No, *n*	Crude OR	95 % CI	Adjusted† OR	95 % CI
Region						
North	209	196	9·47**	6·49, 13·80	9·16**	6·16, 13·63
South	41	364	1·00	ref.	1·00	ref.
Age						
65–69 years	116	290	1·00	ref.	1·00	ref.
70–74 years	134	270	1·24	0·92, 1·67	1·35	0·95, 1·93
Gender						
Male	148	255	1·74*	1·28, 2·35	2·39**	1·50, 3·81
Female	102	305	1·00	ref.	1·00	ref.
Occupation						
Farmer	236	498	2·10**	1·15, 3·83	1·52	0·75, 3·08
Others	14	62	1·00	ref.	1·00	ref.
Educational background						
Illiterate	99	280	1·00	ref.	1·00	ref.
Junior school or higher	151	280	1·53**	1·13, 2·07	1·25	0·84, 1·84
BMI (kg/m^2)						
<24	153	318	1·00	ref.	1·00	ref.
≥24	97	242	0·83	0·61, 1·13	1·05	0·73, 1·50
Hypertension						
Yes	159	310	1·41**	1·04, 1·92	0·99	0·69, 1·42
No	91	250	1·00	ref.	1·00	ref.
Current smoking						
Yes	74	139	1·27	0·91, 1·78	1·00	0·62, 1·62
No	176	421	1·00	ref.	1·00	ref.
Alcohol consumption						
Yes	40	139	0·58**	0·39, 0·85	0·50**	0·30, 0·81
No	209	420	1·00	ref.	1·00	ref.

*$P<0·05$, **$P<0·01$.
†Adjusted for region, gender, age, BMI, occupation, educational background, hypertension, current smoking and alcohol consumption. All variables used in the adjusted model were dichotomous variables.

However, adjustment for the interactions between factors had no important effects on most of these associations except for region, gender and alcohol consumption.

Furthermore, subgroup analyses were conducted in male smokers (because very few women smoked in this population) and in postmenopausal women with HHCY. No significant associations were found in either analysis (data not shown).

Nutritional factors

Finally, we assessed the correlation between Hcy and plasma folate. Overall, plasma Hcy was inversely correlated with plasma folate (Spearman's $r = -0{\cdot}44$, $P < 0{\cdot}001$) as expected. After adjusting for all other factors the inverse relationship still remained, resulting in a significant coefficient (partial correlation $r = -0{\cdot}229$, $P < 0{\cdot}001$). Among subjects with high Hcy levels, deficits of plasma folate were more prevalent in individuals from the north than in those from the south, 33% *v.* 29%, respectively. In contrast, 80% of northerners and 38% of southerners with low plasma folate showed high Hcy levels.

Discussion

High prevalence rate of hyperhomocysteinaemia

To our knowledge, the present study is the first to report data on Hcy concentrations by demographic and lifestyle factors in an elderly Chinese population. We found a high prevalence of HHCY in this population, in particular among the men (61%) living in north China. Notable geographic and gender variations were observed for plasma Hcy concentrations among the study population, which may be the combined results of demographic and lifestyle factors, nutritional status and genetic backgrounds.

Gender, age and alcohol consumption

The largest population-based study, The Hordaland Homocysteine Study, demonstrated that Hcy concentrations increased with age and were higher in men than in women[10]. Detailed data on the Hcy levels of the elderly have also been reported from the third US National Health and Nutrition Examination Survey (NHANES III)[11] and in other studies around the world[12–24]. Our study observed that Hcy levels were higher in men than in women, which is in agreement with the results of the Hordaland study. The male–female differences in Hcy concentrations probably result from the fact that renal cystathionine β-synthase activity is significantly lower in men than women, leading to higher Hcy levels by decreased conversion of Hcy to cysteine in the transsulfuration pathway[25]. However, we did not find significant differences in Hcy concentrations between the two age groups (65–69 *v.* 70–74 years old), perhaps because the age span was smaller than 10 years. Our sample size was large enough that we can exclude the possibility of lacking power for non-significant differences. Additionally, our data confirmed the inverse association between Hcy levels and alcohol consumption reported by the Caerphilly cohort study[26]. The possible explanations include, on the one hand, that the predominant type of alcoholic beverage (i.e. beer) consumed contains folate and that drinkers were likely to consume more food rich in folate (e.g. meat), on the other. These may indirectly improve folate status and decrease Hcy levels.

Regional variations

Overall, the south–north differences observed among the Chinese elderly population are consistent with previous published results from other younger populations. However, it is notable that the absolute prevalence rates of HHCY and the differences in Hcy levels between the two regions were more distinct in the elderly. The larger regional disparities could be explained, in part, by increasing age and different eating habits in the two regions. Moreover, genetic polymorphisms may be another essential factor. It has been reported that persons with the 5,10-methylenetetrahydrofolate reductase (MTHFR) *677-TT* genotype have a 25·7% higher Hcy concentration than those with the *677-CC* genotype[27]. Furthermore, several studies have pointed out that northerners have higher *TT* genotype frequency than southerners[28,29]. Therefore, the *MTHFR C677T* polymorphism may explain, in part, the higher prevalence of HHCY in the north.

In addition, low plasma folate status might account for portions of these variations[30]. Individuals with elevated Hcy concentrations in our study also had low plasma folate concentrations and higher prevalence of deficits, with 10·06 nmol/l and 31·0% in the north *v.* 14·17 nmol/l and 7·7% in the south. We also found that a negative correlation between plasma folate levels and Hcy concentrations existed independent of other demographic and lifestyles factors, suggesting that plasma folate might be an important nutritional factor affecting Hcy levels in the two regions.

Comparison of hyperhomocysteinaemia prevalence between elderly populations in China and developed countries

The NHANES III study (1991–1994) defined HHCY as Hcy level higher than 11·4 μmol/l in male and 10·4 μmol/l in female populations who had good nutritional status and normal renal functioning[11]. On the basis of these criteria, the prevalence of HHCY in our study was converted into 83·7% in the north and 34·3% in the south. These high prevalence rates are twice those in Americans (≥60 years old), which are 43·2% and 46·5% for men and women, respectively. Since 1998, food fortification with folic acid has been implemented in the USA. The latest NHANES study showed that the average Hcy levels of the US population have declined by 8–10% in men and by 3–13% in women. Similarly, Hcy levels of the elderly have changed from 12·5 μmol/l before to 9·8 μmol/l after

fortification[31,32]. These findings suggest that even compared with the American elderly population living 20 years ago, the prevalence of HHCY among Chinese elderly is nearly two times greater, and might be much higher than the current levels of the American elderly.

In a cohort of elderly survivors from the Framingham study population, mean Hcy concentration was 11·9 μmol/l; values higher than 14 μmol/l were detected in 29·3 % of the subjects[33]. Under these criteria, our study found 63·7 % of northerners and 16·3 % of southerners had high Hcy levels.

Recently, a community-based prospective cohort study in Taiwan evaluated the cut-off point of Hcy for predicting risk of vascular diseases and death. Hcy levels were found to be statistically significantly related to cardiovascular events and all-cause deaths, with the best cut-off values being 9·47 μmol/l and 11·84 μmol/l, respectively[34]. Nygard *et al.* previously pointed out that Hcy values ≤9 μmol/l were associated with the lowest mortality level in CVD patients[35]. In our study, 91·1 % of northerners and 59·8 % of southerners were found to be at risk of vascular diseases and death when 9 μmol/l was adopted. This finding implies that the elevated Hcy levels among Chinese elderly populations need to be decreased urgently.

Besides CVD and death, Hcy levels have also been reported to be associated with an increased risk of Alzheimer's disease[36,37] and colon cancer[38] in older individuals. Until ongoing and completed intervention trials with folate in patients with chronic diseases obtain a definite conclusion on the potential roles of HHCY in these diseases, the observed lifestyle–Hcy relationships may contribute to the understanding of the aetiology and to possible prevention of some of these clinical conditions.

One limitation of the present study was the cross-sectional design with blood samples collected only in winter. These made it impossible to evaluate the data in terms of cause-and-effect relationships and we were unable to determine the impact of seasonal factors on Hcy levels. Moreover, our study sample was not a random sample of all the local populations; but on account of the stabilization of the local elderly population as well as their similar dietary habits and lifestyle, they were fairly representative of the eligible target elderly population living in the two regions. However, our study provided strong evidence of a high level of Hcy and a high prevalence of HHCY with distinct regional variations. These data are particularly important in the case of elderly populations who have a high absolute risk of vascular disease, and for whom individualized interventions with folate should be of most benefit.

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