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ORIGINAL RESEARCH

Joint Effects of Plasma Homocysteine Concentration and Traditional Cardiovascular Risk Factors on the Risk of New-Onset Peripheral Arterial Disease

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Purpose: Hyperhomocysteinemia is an independent risk factor for cardio- and cerebrovascular diseases. However, the relationship between plasma homocysteine (Hcy) concentration and peripheral arterial disease (PAD) has not been completely characterized. The aim of the present study was to determine the relationship between plasma Hcy concentration and new-onset PAD and to assess the effects of combinations of Hcy and traditional cardiovascular risk factors.

Patients and Methods: We conducted a prospective community-based cohort study of 3119 Chinese participants who did not have PAD at baseline, with a median follow-up period of 2.30 years. We used multivariate logistic regression models to evaluate the relationship between high Hcy (≥10μmol/L) and new-onset PAD. The effects of combinations of high Hcy and traditional cardiovascular risk factors were assessed using logistic regression analysis.

Results: After adjustment for 14 covariates, high Hcy concentration was significantly associated with new-onset PAD (odds ratio [OR]=2.08, 95% confidence interval [CI]: 1.-08–4.03, *P*=0.030). Smokers with high Hcy concentration were substantially more likely to have new-onset PAD than non-smokers with normal Hcy concentration (OR=4.44, 95% CI: 1.77–11.12, *P*=0.001). The effect of diabetes on PAD became significant when present in combination with high Hcy concentration (OR=3.67, 95% CI: 1.25–10.80, *P*=0.018). Participants with both elevated Hcy levels and older age had the highest risk of new-onset PAD (OR=4.28, 95% CI: 1.83–10.01, *P*<0.001). With regard to the joint effect of Hcy and hypertension, dyslipidemia or sex, there was also a trend towards increased risk across four different groups (*P* for trend=0.026, 0.035, 0.016, respectively).

Conclusion: High plasma Hcy concentration independently predicts the incidence of PAD. Furthermore, there is a joint effect of high Hcy concentration and traditional cardiovascular risk factors such as smoking, diabetes and aging on the incidence of PAD.

Keywords: peripheral arterial disease, atherosclerosis, hyperhomocysteinemia, cohort study, community-based population

Plain Language Summary

Why Was the Study Done?

- Homocysteine (Hcy) is involved in various pathological processes, such as endothelial dysfunction, oxidative stress, and vascular remodeling.
- However, the relationship between high Hcy concentration and peripheral arterial disease (PAD) remains unclear.

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- Moreover, it is also unclear whether Hcy and traditional cardiovascular risk factors have a joint effect on PAD.

What Did the Researchers Do and Find?

- We conducted a prospective community-based cohort study of 3119 Chinese participants who did not have PAD at baseline, with a median follow-up period of 2.30 years.
- In this prospective cohort study, high plasma Hcy concentration was significantly associated with new-onset PAD.
- Moreover, there was a joint effect of high plasma Hcy concentration and traditional cardiovascular risk factors such as smoking, diabetes and aging on the incidence of PAD.

What Do These Results Mean?

- Our study provides new insights into the PAD risk associated with combinations of high Hcy concentration and traditional risk factors.
- Early screening and appropriate treatments are essential among subjects with traditional risk factors accompanying with hyperhomocysteinemia.

Introduction

Peripheral artery disease (PAD) is the term used to describe atherosclerotic occlusion or stenosis of non-cardiac and non-intracranial arteries,[1,2] and it is the third most common manifestation of atherosclerosis after coronary heart disease and stroke.[3] Globally, more than 200 million people have PAD,[4] and its prevalence is expected to increase as the population ages.[5] PAD is strongly associated with a higher risk of cardiovascular events, and it is estimated that there was a 30% increase in the incidences of mortality and disability due to PAD between 2005 and 2015; therefore, it represents a major public health concern.[6,7] A diagnosis of PAD is made when patients have an ankle brachial index (ABI) $\leq 0.90^2$. PAD shares risk factors with other atherosclerotic vascular diseases, with smoking and diabetes being the most potent risk factors.[3] However, evidence for specific risk factors for PAD is less robust than for other atherosclerotic vascular diseases.[8]

In 1969, McCully firstly proposed that the total plasma concentration of homocysteine (Hcy) is high in 15–40% of patients with coronary or cerebral arterial disease, or in individuals with thickened carotid arteries.[9,10] Furthermore, previous studies have shown that hyperhomocysteinemia is an independent and modifiable risk factor for the development of atherosclerosis.[11] Because PAD is one of the manifestations of systemic atherosclerosis, the potential association between Hcy and PAD has been a subject of great interest. However, studies of the relationship between Hcy and PAD have yielded inconsistent results,[12–20] and it requires further characterization in Chinese populations. This is important because there are ethnic disparities in the genetic variations in Hcy metabolic enzymes and lifestyle diversity affecting nutritional status.[8] Moreover, the effects of combinations of hyperhomocysteinemia with traditional cardiovascular risk factors on the prevalence of PAD are also worth exploring. Therefore, the present study was designed to determine the relationship between plasma Hcy concentration and PAD in a Chinese population and to investigate the effects of combinations of hyperhomocysteinemia and traditional cardiovascular risk factors, including hypertension, diabetes, dyslipidemia, smoking, body mass index (BMI), age and sex.

Materials and Methods

Study Population

Participants were recruited from a cohort study of atherosclerosis that included 5962 residents of Beijing, China who were >40 years old, and which was conducted between December 2011 and April 2012. The details of the study procedures have been described previously.[21] Participants were invited for a follow-up examination that took place between May 2014 and July 2014, of whom 3823 (64.1%) attended. The non-responders did not differ substantially from the responders with regard to their baseline characteristics, which were described in detail previously.[21] Patients who had an ABI ≤0.9 at baseline, did not have baseline or follow-up ABI values recorded, or did not have a baseline plasma Hcy concentration recorded were excluded. Consequently, a total of 3119 participants were included in the final analysis (Figure S1). This study was approved by the ethics committee of Peking University, and written informed consent was obtained from each participant. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Data Collection

Baseline data were collected by trained research coordinators using a standard operating procedure. All the participants were interviewed using a standardized questionnaire to collect basic information, including regarding sociodemographic status, lifestyle, and medical history. At baseline, current smoking was defined as smoking at least one cigarette per day for at least half a year and current drinking was defined as drinking alcohol once or more per week for at least half a year. Anthropometric measurements were also

made at baseline according to a standard operating procedure.[21] BMI was calculated as body mass (kg)/height^2 (m^2). Brachial blood pressure (BP) was measured in the right arm of each participant after they had been seated for 5 min using an Omron HEM-7117 electronic sphygmomanometer (Kyoto, Japan), a standard method of calibration, and appropriately sized cuffs. Triplicate measurements were made, with ≥1 min between successive readings, and each patient's systolic BP (SBP) and diastolic BP (DBP) was calculated as the mean of three consecutive measurements. At baseline, hypertension was defined as a self-reported history of hypertension, the use of any antihypertensive drug, an SBP ≥140mmHg, or a DBP ≥90mmHg. Diabetes was defined as a self-reported history, the use of any hypoglycemic drug, a fasting blood glucose (FBG) concentration ≥7mmol/L, or a 2-h blood glucose concentration during oral glucose tolerance testing (OGTT) ≥11.1mmol/L. Dyslipidemia was self-reported or defined as the use of any lipid-lowering drug; or concentrations of triglyceride (TG) ≥1.7mmol/L (150mg/dl), total cholesterol (TC) ≥5.18mmol/L (200mg/dl), low-density lipoprotein-cholesterol (LDL-C) ≥3.37mmol/l (130mg/dl), or high-density lipoprotein-cholesterol (HDL-C) <1.04mmol/L (40mg/dl). Cardiovascular disease (CVD) was defined as a self-reported history of coronary heart disease, stroke, or transient ischemic attack (TIA). To be consistent with previous reports,[22–25] we defined hyperhomocysteinemia as a total plasma Hcy concentration ≥10μmol/L.

Ankle Brachial Index

After having been in a supine position for at least 5 min, each participant's SBP was measured in both arms (the brachial artery) and ankles (the posterior tibial artery) simultaneously using a BP-203RPE III machine (Omron Healthcare). The machine calculated the ABI results automatically, and the left and right ABI values were defined as the ankle SBP on each side, divided by the highest brachial SBP. The overall ABI value was taken to be the lower of the two values obtained. ABI was measured twice during the study: once at baseline and once at the follow-up appointment in May–July 2014. New-onset PAD was defined as the presence of an ABI value ≤0.9 at the follow-up appointment in 2014.

Blood Sample Collection and Laboratory Methods

After an overnight fast of at least 12 h, a venous blood sample was obtained from the forearm of each participant at baseline. Plasma samples were separated within 30 min of collection and were stored at −80°C. Serum samples were used for the measurement of FBG, 2-h blood glucose during a standard 75-g OGTT, TC, LDL-C, HDL-C, TG, and creatinine. Plasma Hcy concentration was measured enzymatically using an automated biochemical analyzer (Beckman Coulter AU480, California, United States). The principle of the method has been described in detail previously.[26] Other laboratory parameters were measured at baseline using a Roche C8000 Automatic Analyzer (Basel, Switzerland).

Statistical Analysis

Continuous variables are reported as median (interquartile range, IQR) and categorical variables are reported as numbers and percentages. The characteristics of the participants and the differences among the participants, classified according to their Hcy concentration (<10 or ≥10μmol/L), were compared using Student's *t*-test for continuous variables or Pearson's chi-square test for categorical variables. For non-normally-distributed data, the Kruskal–Wallis Rank Test was used for continuous variables. Univariate and multivariate logistic regression models were used to determine the relationships of plasma Hcy (as a binary variable) and other variables with the development of new-onset PAD. Two sets of multivariable models were used: Model 1, which was adjusted for baseline ABI, sex, and age; and Model 2, which was further adjusted for BMI, estimated glomerular filtration rate (eGFR) subgroup (eGFR≥90mL/min/1.73m^2, 60≤eGFR<90mL/min/1.73m^2 or eGFR<60mL/min/1.73m^2), current smoking, current drinking, hypertension, diabetes, dyslipidemia, cardiovascular disease, use of an antihypertensive agent, use of a lipid-lowering agent, and use of a hypoglycemic agent (When the odds ratio of new-onset PAD associated with a certain factor is assessed, this factor is not adjusted). To determine the effects of combinations of hyperhomocysteinemia and hypertension on the risk of PAD, we divided the subjects into four different groups according to Hcy (≥10μmol/L/<10μmol/L) and hypertension (yes/no), and compared their risk of PAD to the group with no hypertension and with Hcy concentration <10μmol/L. *P* for trend represents the linear trend across these four different groups. The joint effects of Hcy and diabetes, smoking, or dyslipidemia, BMI, age, sex were assessed in the same way. Interaction and stratified analyses were performed according to chronic disease histories (hypertension, diabetes, or dyslipidemia), smoking status, BMI, age and sex. All analyses were performed using Empower(R) (www.empowerstats.com, X&Y

solutions Boston, MA, USA) and R (http://www.R-project.org). $P < 0.05$ (two-sided) was considered to represent statistical significance.

Results

Baseline Patient Characteristics

The baseline characteristics of all the participants, both overall and stratified according to Hcy concentration, are shown in Table 1. The participants were 56 (51–62) years old; 37.10% were male, and their BMI was 25.87 (23.82–28.03) kg/m^2. Hypertension was present in 49.86% (n = 1555) of the participants, diabetes in 23.85% (n = 744), CVD in 12.63% (n = 394), dyslipidemia in 72.14% (n = 2250), and current tobacco use in 18.95% (n = 591). At the time of enrollment, the median (IQR) Hcy concentration was 11.91 (9.97–14.71) μmol/L. Participants with an Hcy concentration ≥10μmol/L had a significantly higher prevalence of hypertension and CVD, lower eGFR, and were more likely to be taking an antihypertensive drug. Current tobacco and alcohol use were also more frequent in the subgroup of participants with higher plasma Hcy concentration.

Factors Associated with New-Onset PAD

Over a median follow-up period of 2.30 years (25th–75th percentile: 2.30–2.40 years), the incidence of PAD was 3.14% (n = 98). Table 2 displays the results of the multivariate regression analysis of the relationships of Hcy and traditional cardiovascular risk factors with new-onset PAD. An Hcy concentration ≥10μmol/L was associated with a higher risk of new-onset PAD in Model 1 (odds ratio (OR) = 2.21, 95% confidence interval (CI): 1.16–4.21, $P = 0.016$). This relationship remained significant (OR = 2.08, 95% CI: 1.08–4.03, $P = 0.030$) in Model 2. Current smokers were more likely to experience new-onset PAD (OR = 2.20, 95% CI: 1.14–4.22, $P = 0.018$ in Model 2). Diabetes and hypertension seemed to be a risk factor for PAD in univariate regression model (OR = 1.99, 95% CI: 1.31–3.01, $P = 0.001$, and OR = 1.54, 95% CI: 1.02–2.33, $P = 0.039$, respectively), but its influence did not remain significant in Model 1 and 2. The association between dyslipidemia and PAD was not significant ($P = 0.060$ in univariate regression model). Aging was associated with a higher risk of new-onset PAD (OR = 2.15, 95% CI: 1.30–3.55, $P = 0.003$ in full adjusted Model). The association between BMI categories, or sex and PAD was

Table 1 Baseline Characteristics of the Participants

	All (n=3119)	**Hcy<10μmol/L (n=792)**	**Hcy≥10μmol/L (n=2327)**	***P* value**
ABI, median (IQR)	1.11 (1.06–1.16)	1.10 (1.05–1.15)	1.12 (1.06–1.17)	<0.001
Age, median (IQR), years	56 (51–62)	53 (48–57)	57 (52–63)	<0.001
Male, n (%)	1157 (37.10)	83 (10.48)	1074 (46.15)	<0.001
BMI, median (IQR), kg/m^2	25.87 (23.82–28.03)	25.78 (23.53–27.87)	25.89 (23.92–28.08)	0.108
eGFR subgroup, n (%)				<0.001
eGFR ≥ 90mL/min/1.73m^2	2166 (69.49)	699 (88.26)	1467 (63.10)	
60 ≤eGFR<90mL/min/1.73m^2	893 (28.65)	92 (11.62)	801 (34.45)	
eGFR<60mL/min/1.73m^2	58 (1.86)	1 (0.13)	57 (2.45)	
Current smoking, n (%)	591 (18.95)	54 (6.82)	537 (23.08)	<0.001
Current drinking, n (%)	721 (23.12)	90 (11.36)	631 (27.12)	<0.001
Prevalence of disease				
Hypertension, n (%)	1555 (49.86)	342 (43.18)	1213 (52.13)	<0.001
Diabetes, n (%)	744 (23.85)	179(22.60)	565 (24.28)	0.338
Dyslipidemia, n (%)	2250 (72.14)	571 (72.10)	1679 (72.15)	0.975
CVD, n (%)	394 (12.63)	77 (9.72)	317 (13.62)	0.004
Medication				
Antihypertensive drugs, n (%)	990 (31.94)	221 (28.08)	769 (33.25)	0.007
Hypoglycemic drugs, n (%)	310 (9.97)	78 (9.87)	232 (10.00)	0.916
Lipid-lowering drug, n (%)	322 (10.43)	86 (11.04)	236 (10.23)	0.523

Abbreviations: Hcy, homocysteine; ABI, ankle brachial index; BMI, body mass index; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; IQR, interquartile range.

Table 2 Odds Ratio for New-Onset PAD Associated with High Hcy Concentration and Traditional Cardiovascular Risk Factors

Factor	Crude		Model 1[a]		Model 2[b]	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Hcy≥10μmol/L	2.49 (1.36–4.59)	0.003	2.21 (1.16–4.21)	0.016	2.08 (1.08–4.03)	0.030
Hypertension	1.54 (1.02–2.33)	0.039	1.34 (0.86–2.11)	0.197	1.19 (0.63–2.25)	0.592
Diabetes	1.99 (1.31–3.01)	0.001	1.45 (0.93–2.27)	0.099	1.28 (0.70–2.32)	0.424
Dyslipidemia	1.63 (0.98–2.70)	0.060	1.35 (0.80–2.27)	0.264	1.12 (0.65–1.93)	0.688
Current smoking	1.32 (0.82–2.13)	0.247	2.11 (1.14–3.90)	0.017	2.20 (1.14–4.22)	0.018
BMI, kg/m^2						
<24	Ref.		Ref.		Ref.	
≥24, <28	0.88 (0.54–1.46)	0.630	0.88 (0.53–1.47)	0.624	0.87 (0.51–1.49)	0.609
≥28	1.28 (0.76–2.18)	0.356	0.99 (0.57–1.73)	0.982	0.95 (0.53–1.71)	0.868
Age≥60 years	2.66 (1.78–3.99)	<0.001	3.22 (2.12–4.88)	<0.001	2.15 (1.30–3.55)	0.003
Female	1.17 (0.76–1.79)	0.477	0.97 (0.62–1.52)	0.907	1.36 (0.74–2.50)	0.322

Notes: [a]Model 1: adjusted for baseline ABI, sex, and age. [b]Model 2: adjusted for baseline ABI, sex, age, body mass index, estimated glomerular filtration rate subgroup, current smoking and drinking, hypertension, diabetes, dyslipidemia, cardiovascular disease, and the use of antihypertensive, lipid-lowering, or hypoglycemic agents. (When the odds ratio of new-onset PAD associated with a certain factor is assessed, this factor is not adjusted. For example, dyslipidemia is not adjusted when the odds ratio of new-onset PAD associated with dyslipidemia is assessed).

Abbreviations: Hcy, homocysteine; OR, odds ratio; CI, confidence interval; PAD, peripheral artery disease; ABI, ankle brachial index; BMI, body mass index.

not significant. Interaction analysis showed no interaction effect according to different traditional cardiovascular risk factors (Table S1).

The ORs for new-onset PAD for the various combinations of Hcy and traditional risk factors are displayed in Table 3 and Figure 1. Assessment of the effect of high Hcy in combination with smoking revealed a joint effect. Compared with non-smoking participants with normal Hcy concentration, the OR for incident PAD increased by a factor of 2.08 for participants with high Hcy and by a factor of 2.10 for smokers. Smokers with a high Hcy concentration had an OR for incident PAD of 4.44, corresponding to a multiplicative relationship on the odds scale. Although the association between diabetes and PAD in participants with a Hcy concentration <10μmol/L was not significant, there was a significant association of a combination of Hcy concentration >10μmol/L and the presence of diabetes with PAD risk (OR = 3.67, 95% CI: 1.25–10.80, $P = 0.018$). This effect was stronger than that of Hcy alone in non-diabetic subjects. Also, there were joint effects of high Hcy concentration and aging on the incidence of PAD. Participants with both elevated Hcy levels and older age had the highest risk of new-onset PAD (OR = 4.28, 95% CI: 1.83–10.01, $P <0.001$).

Among the four subgroups, hypertensive patients with hyperhomocysteinemia had the highest risk of incident PAD but were not statistically significant compared with non-hypertensives with normal Hcy concentration (OR = 1.99, 95% CI: 0.75–5.27, $P = 0.166$). However, there seemed to be a trend towards increased risk across the four subgroups (P for trend=0.026). The joint effect of high Hcy concentration and dyslipidemia on new-onset PAD also existed (P for trend=0.035). With regard to the joint effect of Hcy and sex, there was also a trend towards increased risk across four different groups (P for trend=0.016), females with hyperhomocysteinemia had the highest risk of incident PAD but was not statistically significant compared with males with Hcy<10μmol/L (OR = 2.79, 95% CI: 0.35–22.11, $P = 0.330$). However, there seemed no joint effect of high Hcy concentration and BMI.

Discussion

The major findings of the present study were as follows. A plasma Hcy concentration of ≥10μmol/L was independently associated with a higher incidence of PAD in a community-based cohort in China. Although participants with higher plasma Hcy concentration tended to be older and have more traditional risk factors for CVD, such as hypertension and a smoking habit, than those with lower concentrations, the association remained significant after adjusting for these risk factors. Furthermore, high Hcy concentration and traditional cardiovascular risk factors such as smoking, diabetes and aging had a joint effect on the incidence of PAD. Our results extend previous findings and suggest that hyperhomocysteinemia and some traditional risk factors have joint effects on PAD risk. Thus, the assessment of plasma Hcy concentration alongside

Table 3 Odds Ratio for New-Onset PAD Associated with the Various Combinations of High Hcy Concentration and Traditional Cardiovascular Risk Factors

Subgroups		Incidence, n(%)	OR (95% CI) [a]	*P* for effect	*P* for trend
Hcy × Hypertension					0.026
Hcy<10μmol/L	Hypertension=no	7 (1.56)	Ref.		
Hcy<10μmol/L	Hypertension=yes	5 (1.46)	0.68 (0.19–2.44)	0.553	
Hcy≥10μmol/L	Hypertension=no	32 (2.87)	1.52 (0.64–3.57)	0.343	
Hcy≥10μmol/L	Hypertension=yes	54 (4.45)	1.99 (0.75–5.27)	0.166	
Hcy × Diabetes					0.020
Hcy<10μmol/L	Diabetes=no	5 (0.82)	Ref.		
Hcy<10μmol/L	Diabetes=yes	7 (3.91)	2.96 (0.83–10.55)	0.094	
Hcy≥10μmol/L	Diabetes=no	56 (3.18)	3.20 (1.23–8.31)	0.017	
Hcy≥10μmol/L	Diabetes=yes	30 (5.31)	3.67 (1.25–10.80)	0.018	
Hcy × Dyslipidemia					0.035
Hcy<10μmol/L	Dyslipidemia=no	1 (0.45)	Ref.		
Hcy<10μmol/L	Dyslipidemia=yes	11 (1.93)	3.28 (0.41–26.32)	0.263	
Hcy≥10μmol/L	Dyslipidemia=no	18 (2.78)	5.83 (0.75–45.49)	0.092	
Hcy≥10μmol/L	Dyslipidemia=yes	68 (4.05)	5.72 (0.76–42.85)	0.090	
Hcy × Smoking					0.003
Hcy<10μmol/L	Smoking=no	11 (1.49)	Ref.		
Hcy<10μmol/L	Smoking=yes	1 (1.85)	2.10 (0.25–17.44)	0.494	
Hcy≥10μmol/L	Smoking=no	64 (3.58)	2.08 (1.04–4.14)	0.038	
Hcy≥10μmol/L	Smoking=yes	22 (4.10)	4.44 (1.77–11.12)	0.001	
Hcy × BMI					
Hcy<10μmol/L	BMI<24 kg/m^2	1 (0.41)	Ref.		0.077
Hcy<10μmol/L	24≤BMI<28 kg/m^2	6 (1.67)	4.67 (0.55–39.70)	0.158	
Hcy<10μmol/L	BMI≥28 kg/m^2	5 (2.66)	4.56 (0.51–40.52)	0.174	
Hcy≥10μmol/L	BMI<24 kg/m^2	25 (4.20)	9.33 (1.22–71.16)	0.031	
Hcy≥10μmol/L	24≤BMI<28 kg/m^2	35 (3.09)	6.45 (0.86–48.54)	0.071	
Hcy≥10μmol/L	BMI≥28 kg/m^2	26 (4.34)	7.18 (0.94–54.74)	0.057	
Hcy × Age					<0.001
Hcy<10μmol/L	Age<60 years	8 (1.23)	Ref.		
Hcy<10μmol/L	Age≥60 years	4 (2.88)	1.87 (0.52–6.69)	0.334	
Hcy≥10μmol/L	Age<60 years	37 (2.49)	2.07 (0.93–4.62)	0.076	
Hcy≥10μmol/L	Age≥60 years	49 (5.83)	4.28 (1.83–10.01)	<0.001	
Hcy × Sex					0.016
Hcy<10μmol/L	Male	1 (1.20)	Ref.		
Hcy<10μmol/L	Female	11 (1.55)	1.32 (0.16–11.15)	0.797	
Hcy≥10μmol/L	Male	32 (2.98)	1.80 (0.23–13.78)	0.572	
Hcy≥10μmol/L	Female	54 (4.31)	2.79 (0.35–22.11)	0.330	

Notes: [a]Adjusted for baseline ABI, sex, age, body mass index, estimated glomerular filtration rate subgroup, current smoking and drinking, hypertension, diabetes, dyslipidemia, cardiovascular disease, and the use of antihypertensive, lipid-lowering, or hypoglycemic agents. (When the odds ratio of new-onset PAD associated with the combination of high Hcy concentration and a certain factor is assessed, this factor is not adjusted. For example, dyslipidemia is not adjusted when the odds ratio of new-onset PAD associated with the combination of high Hcy concentration and dyslipidemia is assessed).

Abbreviations: Hcy, homocysteine; OR, odds ratio; CI, confidence interval; PAD, peripheral artery disease; ABI, ankle brachial index; BMI, body mass index.

traditional cardiovascular risk factors may improve PAD risk stratification.

The median plasma Hcy concentration at baseline was similar to that reported from previous large-scale studies conducted in Chinese populations (median 12.5μmol/L, IQR 10.5–15.5μmol/L),[27] but was higher than that reported from the Framingham study[28] and those reported in other countries.[29] This might be attributable to the high

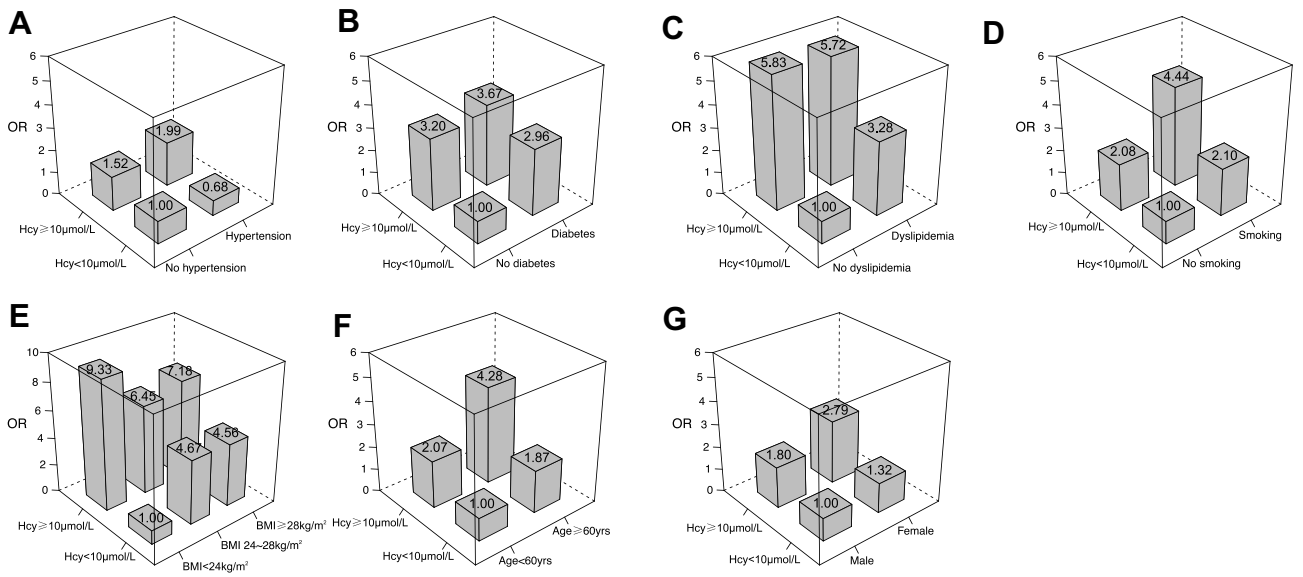

Figure 1 Odds ratio for new-onset PAD associated with the combinations of high Hcy concentration and hypertension (**A**), diabetes (**B**), dyslipidemia (**C**), smoking (**D**), BMI (**E**), Age (**F**) and Sex (**G**)[a].

Notes: [a]Adjusted for baseline ABI, sex, age, body mass index, estimated glomerular filtration rate subgroup, current smoking and drinking, hypertension, diabetes, dyslipidemia, cardiovascular disease, and the use of antihypertensive, lipid-lowering, or hypoglycemic agents. (When the odds ratio of new-onset PAD associated with the combination of high Hcy concentration and a certain factor is assessed, this factor is not adjusted. For example, dyslipidemia is not adjusted when the odds ratio of new-onset PAD associated with the combination of high Hcy concentration and dyslipidemia is assessed).

Abbreviations: Hcy, homocysteine; OR, odds ratio; PAD, peripheral artery disease; ABI, ankle brachial index; BMI, body mass index.

frequency of the 677TT genotype of the methylene tetrahydrofolate reductase (*MTHFR*) gene in the Chinese population[30–32] and the different diets and nutritional status in China.[33] Therefore, it is extremely important to focus on the role of Hcy in the Chinese population. Previous studies have suggested that individuals with an Hcy ≥10μmol/L are at a higher risk of cardio- and cerebrovascular events, both in China[34] and the U.S.[35] Therefore, and in accordance with previous reports,[24] we selected 10μmol/L as the cut-off value when assessing the impact of Hcy concentration in the present study.

In 1995, a meta-analysis of three case–control studies conducted by Boushey et al showed that Hcy was a risk factor for PAD (OR=6.8, 95% CI 2.9–15.8).[17] Then, in 2009, another meta-analysis of 14 case–control studies demonstrated that Hcy concentration in patients with PAD was 4.31μmol/L higher than that of controls without PAD.[18] A recent case–control study conducted in a Chinese population also indicated that Hcy was associated with PAD, independent of classical vascular risk factors.[20] However, prospective studies have yielded conflicting results. Ridker et al performed a nested case–control study using samples from the Physicians' Health Study cohort, but no association was found between Hcy and PAD.[12] In 2006, Aboyans et al investigated the relationship between Hcy concentration and PAD progression, but found no association.[13] Finally, Monica et al conducted a nested case–control study that showed that Hcy concentration was positively associated with the risk of PAD in men, but not in women.[15] In the present study, we followed a cohort of individuals who did not have PAD at baseline, and have shown that high plasma Hcy concentration is an independent risk factor for new-onset PAD. The main reason why our results are inconsistent with previous cohort studies may be the population differences. In the majority of Western populations, folic acid fortification was implemented and their Hcy levels were lower than that of the Chinese population.[28] The relative lower Hcy level may lead to a negative result in their studies.[12] Laboratory research has shown that Hcy is involved in various pathological processes, such as endothelial dysfunction, oxidative stress, and vascular remodeling,[33] and these mechanisms may explain how Hcy increases the risk of PAD. Since the deleterious effect of Hcy on cardio- and cerebrovascular diseases is now well established, our findings have further elucidated its effect on PAD risk.

Current smoking was found to be associated with an increased risk of new-onset PAD, which was consistent with previous studies.[3] However, the definition of current smoking was based on self-reported smoking habits,

measurement of plasma cotinine levels might be needed in the future studies to reflect the smoking status more precisely. Perhaps surprisingly, the effect of diabetes on PAD risk was not significant in the present study. As the outcome of our study was new-onset PAD, subjects with PAD at baseline were excluded. The mechanism of diabetes causing PAD lies in endothelial dysfunction with denervation of the vascular smooth muscle cells, which is a relatively long process. Moreover, the duration of diabetes was found to be associated with the risk of developing PAD, so it might be difficult to show a significant effect of diabetes within 2 to 3 years.[36] Hypertension and dyslipidemia on PAD risk were also not significant. Although PAD is associated with similar risk factors to other atherosclerotic diseases, the effects of hypertension and dyslipidemia on PAD are modest.[37] A study of 7058 people of ≥40 years of age from NHANES showed that the OR for PAD associated with hypertension was 1.5 (95% CI: 0.9–2.2, $P = 0.05$).[38] Another study conducted in a multi-ethnic Asian population comprising 4132 participants demonstrated that those with PAD had higher SBP, but the association was lost in multivariate analysis. Furthermore, there was no association between dyslipidemia and PAD.[39] It was established that the incidence of PAD increased with aging,[37] which was consistent with our results. The effect of sex on PAD has been controversial, as previous evidence showed an increased prevalence of PAD in male, but some recent studies found PAD was more common in female.[40,41] Recent global estimates found prevalence of PAD to be the same among gender in high-income countries, but more prevalent in women in low- or middle-income countries like China.[3] In our study, no significant association was observed between sex and incident PAD but the trend towards the same direction.

Previous studies have shown that traditional risk factors have joint effects on the risk of PAD when present in combination. Joosten et al performed a prospective study of 44,985 men, with a median follow-up period of 24.2 years, and identified joint effects of combinations of a smoking habit, hypertension, hypercholesterolemia, and type 2 diabetes.[42] Furthermore, Eraso et al used data from the National Health and Nutrition Examination Survey (NHANES) and included hypertension, diabetes, chronic kidney disease, and smoking as PAD risk factors. The risk of PAD increased with each additional risk factor present, from a non-significant 1.5-fold increase (OR=1.5, 95% CI: 0.9–2.6) in the presence of one risk factor, to a > 10-fold increase (OR=10.2, 95% CI: 6.4–16.3) in the presence of three or more risk factors.[38] However, until now, the effects of combinations of hyperhomocysteinemia and traditional risk factors on PAD risk were largely unknown, our results filled this gap. Our study suggests that special attention is needed in hyperhomocysteinemia in subjects with traditional risk factors such as smokers, elder and patients with diabetes, since they are more likely to suffer from PAD in the future. Early screening and appropriate treatments are essential.

The present study had several limitations. First, study subjects were recruited from a single community in Beijing; therefore, the data might not be representative of populations in other locations in China. The population differences in plasma Hcy concentration might make it necessary to be cautious when generalizing our findings to other populations. Second, the time scale for the development of PAD should be determined more accurately, because we only followed up the participants at two time points. Third, because of the relatively short follow-up period, the incidence of new-onset PAD in the sample was relatively low, which may have affected the results of the subgroup analyses. Therefore, larger studies with longer follow-up times should be conducted to verify the effect of plasma Hcy concentration on PAD and its relationships with other traditional cardiovascular risk factors, which may improve risk prediction and assist with the identification of groups at high risk of PAD in the future.

To our knowledge, this is the largest longitudinal study to date to demonstrate that hyperhomocysteinemia is a significant predictor of PAD in a Chinese population. Because the distribution of genetic polymorphisms of Hcy metabolic enzymes and dietary pattern differs geographically, it is important to provide evidence for distinct populations, including the Chinese population. The present cohort study is also the first to investigate the joint effects of hyperhomocysteinemia and conventional cardiovascular risk factors on PAD risk in China. Although the relatively short follow-up period was a limitation, the present study provides new insight into integrating the effect of hyperhomocysteinemia into certain traditional risk factors on PAD risk.

Conclusions

In conclusion, high plasma Hcy independently predicted new-onset PAD in a Chinese community-based population. There is a joint effect of high Hcy concentration and traditional cardiovascular risk factors such as smoking, diabetes and aging on the incidence of PAD. Therefore,

early screening and appropriate treatments are crucial among subjects with traditional risk factors when accompanying with hyperhomocysteinemia.

Abbreviations

Hcy, homocysteine; PAD, peripheral arterial disease; ABI, ankle brachial index; BMI, body mass index; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; OGTT, oral glucose tolerance testing; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CVD, cardiovascular disease; TIA, transient ischemic attack; SD, standard deviation; eGFR, estimated glomerular filtration rate; IQR, interquartile range; OR, odds ratio; CI, confidence interval; MTHFR, methylene tetrahydrofolate reductase; NHANES, National Health and Nutrition Examination Survey.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The datasets intended for sharing have been deidentified.

Ethics Approval and Informed Consent

This study was approved by the ethics committee of Peking University (approval numbers: IRB00001052-11086), and written informed consent was obtained from each participant. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

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Author Contributions

All authors reviewed and approved the manuscript, and they agree to be accountable for all aspects of the work. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed on the journal to which the article will be submitted; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest for this work.

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