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Plasma homocysteine levels are independently associated with alterations of large artery stiffness in men but not in women

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Abstract

Objectives To investigate the associations of the plasma homocysteine levels with the alterations in arterial stiffness in a community-based cohort. The gender differences in these associations were examined. **Methods** We evaluated the relationship between plasma homocysteine levels to three measures of vascular function [carotid-femoral pulse wave velocity (CF-PWV), carotid-ankle PWV (CA-PWV) and heart rate corrected augmentation index (AI)] in 1680 participants (mean age: 61.5 years; 709 men, 971 women) from communities of Beijing, China. **Results** In univariate analysis, plasma homocysteine levels was positively related to the CF-PWV ($r = 0.211$, $P < 0.0001$) and CA-PWV ($r = 0.148$, $P < 0.0001$), whereas inversely associated with AI ($r = –0.052$, $P = 0.016$). In multiple linear regression models adjusting for covariants, plasma homocysteine remained positively related to the CF-PWV (standardized $\beta = 0.065$, $P = 0.007$) in total cases. When the groups of men and women were examined separately, plasma homocysteine remained positively associated with the CF-PWV (standardized $\beta = 0.082$, $P = 0.023$) in men, whereas the relations between homocysteine and any of the arterial stiffness indices were not further present in women. **Conclusions** In Chinese population, plasma homocysteine levels are independently associated with alterations of large artery stiffness in men but not in women.

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Keywords: Arterial stiffness; Gender differences; Homocysteine; Pulse wave velocity

1 Introduction

Cardiovascular disease is the biggest burden and dominant chronic disease in the world.[1] Increased arterial stiffness and higher plasma homocysteine levels are related to elevated risk for cardiovascular disease. It has been reported that arterial pulse pressure, an indirect indicator of aortic arterial stiffness, is a predictor of cardiovascular events.[2–4]

The development of methods to measure and assess specific aspects of arterial stiffness greatly facilitated understanding of its role in cardiovascular disease. Pulse wave velocity (PWV) measurement is performed by determining the time delay between two sites in the line of pulse travel and the distance between the sites, with which it is possible to assess the stiffness in distinct part of the arterial tree.[5] There are increasing evidences of the relationship between cardiovascular risk and arterial stiffness, including carotid-femoral PWV (CF-PWV) and carotid-ankle PWV (CA-PWV), and the augmentation index (AI).[6–8]

Stiffness of artery increases with age and is associated with traditional vascular risk factors.[9–11] The increases in central and peripheral arterial stiffness are proposed to be related to several mechanical and biological factors, including "extrinsic factors" and hemodynamic forces from diverse pathways.[12,13] Epidemiological data have shown that plasma homocysteine is more strongly associated with systolic than with diastolic blood pressure.[14] Therefore, it has been hypothesized that hyperhomocysteinemia increases arterial stiffness. Previous studies have investigated the associations of measures of arterial stiffness with levels of plasma homocysteine.[15–20] However, the conclusions of these studies are inconsistent. In addition, the associations of homocysteine with arterial stiffness, especially the gender differences in these associations, have not been assessed in large sample of Chinese population.

The aim of the present study is to explore whether homocysteine is associated with arterial stiffness and to investigate the gender differences in the associations of homo-

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cysteine with arterial stiffness in Chinese population. To test this hypothesis, we related plasma homocysteine levels to 3 vascular function measures PWV (CF-PWV, CA-PWV, and AI) in a large community-based sample of China.

2 Methods

2.1 Study population

This was a community-based cross-sectional study of people living in Pingguoyuan area in Beijing, China. After a routine health check-up between September 2007 and January 2009, initially, a total of 1859 permanent residents were recruited to the study as described previously.[21] Thirty one subjects with severe systemic diseases such as collagenosis, endocrine and metabolic disease [except diabetes mellitus (DM)], inflammation, neoplastic or severe liver or renal diseases were excluded from the analysis.

Arterial stiffness assessment was attempted to be performed in 1828 subjects. Adequate measurement was either not obtained or not attempted in the 86 participants. Another 37 participants were excluded because of missing data on plasma HOMOCYSTEINE or other measurements. In addition, 25 participants were excluded because of missing information for multivariable analyses. Thus, 1680 participants were eligible for analysis. The study was approved by the ethics committee of Chinese PLA General Hospital, and each participant provided written informed consent.

2.2 Clinical data collection

Each subjects completed a standardized form that included family history of CVD, medical history and lifestyle. The physical interview and examination were performed by trained nurses and physicians. Two recordings of blood pressure were obtained from the right arm of subjects; measurements were taken in 5-min intervals, then mean values were calculated. Hypertension was defined as a mean of three independent measures of blood pressure $\geq$ 140/90 mmHg or current use of antihypertensive drugs. Diabetes mellitus (DM) was diagnosed when the subject had a fasting glucose $\geq$ 7.8 mmol/L, $\geq$ 11.1 mmol/L at 2 h after oral 75 g glucose challenge, or both, or current use of antidiabetic agents. The categories of smoking included current smoking, former smoking and never smoking. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.3 Biochemical variable determination

All participants underwent full laboratory evaluation. Blood samples were obtained between 8 a.m. and 10 a.m from fasting subjects, centrifuged immediately, and stored at −80°C until assays were performed. The biochemical variables were measured with an automatic analyzer (Roche Cobas e601, Swaziland).

2.4 Homocysteine detection

Plasma levels of homocysteine were measured about two days around the artery stiffness assessment for the present analyses. Homocysteine was assayed by high-performance chromatography with fluorometric detection. The inter-assay variationsfor homocysteine was 4.1%.

2.5 Assessment of arterial stiffness

Participants were studied in the supine position after resting 5 to 10 min. Arterial stiffness was evaluated by automatic CF-PWV and CA-PWV measurements using the Complior Colson device (Createch Industrie, France); the technical characteristics of this device have been described previously.[22] PWV along the artery was measured by using two strain-gauge transducers [using a TY-306 Fukuda pressure-sensitive transducer (Fukuda Denshi Co, Japan)] fixed transcutaneously over the course of a pair of arteries separated by a known distance; the carotid, femoral, and ankle arteries (all on the right side) were used. Measurements were repeated over 10 different cardiac cycles, and the mean values were used for the final analysis. PWV was calculated from the measurements of the pulse transit time and the distance traveled by the pulse between the two recording sites (measured on the surface of the body in meters), according to the following formula: PWV (m/s) = distance (m)/transit time (s).

AI is a composite measure of the magnitude of wave reflection and arterial stiffness, which affects the timing of wave reflection.[23] To take into account the potential effect of heart rate on AI, all values were automatically corrected by heart rate.

2.6 Statistical analyses

The arterial stiffness measures included in analyses were CF-PWV, CA-PWV and heart rate corrected AI. Baseline characteristics and arterial variables were tabulated separately for men and women. Levels of homocysteine were natural logarithmically transformed to normalize their distributions. Relations between homocysteine with arterial stiffness measures were described using Pearson's correlation coefficients. Sex-specific multiple linear regression analyses were conducted to examine whether simple associations were changed after adjustment for potential confounders or intermediaries. The multiple linear regression models adjusted for the following covariates: age, age square and clinical covariates, including sex, weight, height,

plasma total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, blood glucose, current smoking, heart rate, and mean arterial pressure. Analyses were performed with Stata software (version 11.0; Stata Corporation, College Station, TX). A two-sided value of $P < 0.05$ was considered significant.

3 Results

A total of 1680 subjects (mean age: 61.5 years; 709 men, 971 women) were eligible for analyses. Clinical characteristics of the study sample, values of biomarkers and mean arterial stiffness values are presented in Table 1, grouped by sex.

Correlation coefficients obtained in the Pearson correlation analysis between plasma homocysteine and arterial stiffness measures (CF-PWV, CA-PWV and AI) in the total population were shown in Table 2. Homocysteine was positively related to the CF-PWV ($r = 0.211$, $P < 0.0001$) and CA-PWV ($r = 0.148$, $P < 0.0001$), whereas inversely associated with AI ($r = –0.052$, $P = 0.016$).

Table 1. Characteristics of the study sample.

Characteristics	Men (n = 709)	Women (n = 971)
Age, yrs	62.09 ± 11.35	61.11 ± 10.81
BMI, kg/m^2	25.46 ± 3.21	25.53 ± 3.87
Waist circumference, cm	89.88 ± 9.29	85.00 ± 10.05$^{\#}$
SBP, mm Hg	134.48 ± 18.64	128.74 ± 18.45$^{\#}$
DBP, mm Hg	78.88 ± 10.79	75.01 ± 10.31$^{\#}$
Heart rate, beats/min	74.86 ± 10.15	75.74 ± 9.78
TC, mmol/L	4.90 ± 0.89	5.17 ± 0.93$^{\#}$
TG, mmol/L	1.47 (1.07, 2.17)	1.47(1.10, 2.08)
HDL-C, mmol/L	1.29 ± 0.33	1.46 ± 0.38$^{\#}$
LDL-C, mmol/L	2.85 ± 0.71	3.07 ± 0.73$^{\#}$
Fasting glucose, mmol/L	5.46 ± 1.14	5.43 ± 1.79
120 min postprandial glucose, mmol/L	7.51 ± 4.0	8.08 ± 4.22$^{\#}$
Homocysteine, μmol/L	19.4 (15.5, 24.9)	15.8 (13, 19.2)$^{\#}$
Carotid-femoral PWV, m/s	11.89 ± 3.24	11.34 ± 2.94$^{\#}$
Carotid-ankle PWV, m/s	9.53 ± 2.00	9.18 ± 1.77$^{\#}$
Augmentation index†	23.1% ± 9.4%	23.3% ± 9.1%
Current smoking	46.6%	8.4%$^{\#}$
Diabetes	20.9%	20.8%
Hypertension	56.4%	51.0%*

Data are presented as mean ± SD or median (quartile 1, quartile 3) unless other indicated. BMI: body mass index; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PWV: pulse wave velocity; SBP: systolic blood pressure; TC: total plasma cholesterol; TG: triglycerides. *$P < 0.05$ and $^{\#}P < 0.01$ compared with the men group. †Corrected for heart rate.

Table 2. Correlations between homocysteine and arterial stiffness measures in total subjects.

Parameters of arterial stiffness	Homocysteine	
	r*	P
Carotid-femoral PWV, m/s	0.211	< 0.0001
Carotid-ankle PWV, m/s	0.148	< 0.0001
Augmentation index, %	–0.052	0.016

*Data are r values of Pearson's correlation coefficients between parameters. PWV: pulse wave velocity.

The results of multiple linear analyses were shown in Table 3. In multiple-adjusted models, homocysteine levels remained a significant determinant of the CF-PWV (standardized $\beta = 0.065$, $P = 0.007$). According to demographic and clinical indices, age and mean arterial pressure were most important influencing factors on the arterial stiffness measures.

When the groups of men and women were examined separately, plasma homocysteine remained positively associated with the CF-PWV (standardized $\beta = 0.082$, $P = 0.023$) in men, whereas were not significantly related to any of the arterial stiffness measures in women. In addition, age and mean arterial pressure remained a significant determinant of several measures of arterial stiffness both in men and women.

4 Discussion

This is the first study to investigate the gender differences in the association between plasma homocysteine and several indexes of arterial stiffness in a large community-based sample in China. The plasma homocysteine concentration was positively related to CF-PWV. In men but not in women, homocysteine was independently associated with aortic artery stiffness even after controlling for age and other conventional cardiovascular risk factors. As for demographic and clinical features, age, sex and blood pressure emerged as most important influencing factors on the arterial stiffness measures.

Assessment of arterial waveforms includes carotid-femoral (elastic), carotid-radial (muscular), and carotid-ankle (composite measure of elastic and muscular arterial tree) PWV, and the AI, a composite measure of systemic arterial stiffness and wave-reflection amplitude. In particular, the velocity of the carotid-femoral or aortic pulse wave appears to be of prognostic importance and is considered to be the "gold standard" for central artery stiffness.[24] In the present study, measure of CA-PWV and AI were not associated with homocysteine levels on multiple analyses, indicating that plasma homocysteine levels were independently associated with alterations of central artery stiffness but not peripheral stiffness and wave-reflection amplitude.

Table 3. Predictors of measures of arterial stiffness: results of multiple-adjusted* models.

Parameters	R^2 for model	Global *P*	Standardized β	*P*
Total population, *n* = 1680				
Carotid-femoral PWV, m/s	0.5735	< 0.001		
Homocysteine			0.065	0.007
Age			0.498	< 0.0001
Height			0.048	0.038
Mean arterial pressure			0.112	< 0.0001
Glucose			0.102	< 0.0001
Heart rate			0.075	0.0007
HDL-C			–0.051	0.023
Carotid-ankle PWV	0.4408	< 0.001		
Age			1.293	< 0.0001
Female gender			–0.079	0.002
Mean arterial pressure			0.189	< 0.0001
Heart rate			0.1052	< 0.0001
Augmentation index	0.4249	< 0.001		
Age			0.757	0.0001
Height			–0.272	< 0.0001
Mean arterial pressure			0.088	0.0003
Men, *n* = 709				
Carotid-femoral PWV	0.5203	< 0.001		
Homocysteine			0.082	0.023
Age			0.459	< 0.0001
Mean arterial pressure			0.157	< 0.0001
Heart rate			0.103	0.004
Carotid-ankle PWV	0.3525	< 0.001		
Age			1.234	< 0.0001
Mean arterial pressure			0.156	0.0001
TC			0.090	0.021
Augmentation index	0.3832	< 0.001		
Age			1.102	0.0005
Height			–0.243	< 0.0001
Mean arterial pressure			0.079	0.039
Women, *n* = 971				
Carotid-femoral PWV	0.6061	< 0.001		
Age			0.534	< 0.0001
Mean arterial pressure			0.075	0.008
Glucose			0.121	< 0.0001
HDL-C			–0.072	0.012
Heart rate			0.058	0.039
Carotid-ankle PWV	0.5155	< 0.001		
Age			1.145	< 0.0001
Mean arterial pressure			0.216	< 0.0001
Heart rate			0.137	< 0.0001
Glucose			0.137	< 0.0001
Augmentation index	0.2460	< 0.001		
Height			–0.180	< 0.0001
Mean arterial pressure			0.133	< 0.0001
Current smoking			0.071	0.034

Stepwise multiple linear regression analysis is performed in total population, and separately in Men and Women. *Covariates in the multiple-adjusted models included age, age square, sex, height, weight, heart rate, TC, HDL-C, LDL-C, current smoking, blood glucose, and mean arterial pressure. Standardized β provides a measure of the relative strength of the association independent of the measurement units. Standardized β and *P* value are only shown when $P < 0.05$. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PWV: pulse wave velocity; TC: total plasma cholesterol.

Previous studies have investigated relations of homocysteine and different indices of arterial stiffness.[16,17,25,26] Most of these studies had modest sample sizes. Tayama, *et al*.[26] have found that higher plasma homocysteine concentration was independently associated with greater brachial-ankle PWV in 50 hypertensive patients. In studies with large sample size, the conclusions are also inconsistent.[19,20] The different results in these studies can be partly explained by the fact that hyperhomocysteinaemia may interact with other cardiovascular risk factors, particularly, age, sex and blood pressure.

In the present study, based on a large population-based sample from Beijing, China, we observed a consistent positive association of homocysteine with aortic (carotid-femoral) PWV in men, whereas no statistical significant association was found between homocysteine and any of the arterial stiffness measures in women. These findings observed in this study are in line with those of a prior Framingham publication,[19] that reported an association of homocysteine with aortic artery stiffness in men by not in women, indicating a potential role of homocysteine in remodelling of the arterial wall leading to arterial stiffness in men.

The gender differences in the relationship between plasma homocysteine and aortic stiffness are intriguing, but the mechanism is not fully understood. In the present study, plasma homocysteine levels were significantly higher in men than in women, indicating that the significant positive association of homocysteine with aortic stiffness may be related to higher homocysteine concentrations in men. In addition, it is possible that differences in homocysteine metabolism between the sexes could partially explain this phenomenon. Homocysteine, which is produced by the transmethylation of methionine, can be either remethylated back to methionine or metabolized via transsulfuration to cystathionine. It has been believed that more rapid cycling in women resulted in a greater proportion of homocysteine being diverted to cystathionine.[27] Blom, *et al*.[28] found that higher rate of methionine transamination in women may contribute to lower homocysteine concentrations and hence protect against vascular disease. Additional studies are needed to verify the findings of the present study and to further investigate the mechanisms of the gender differences in the association of homocysteine with aortic stiffness.

Several limitations of the present study need to be considered. First, because of the cross-sectional design and its inherent limitations, the present study cannot determine causal relationships between the associations. Longitudinal and interventional studies should be performed to establish the directionality of these relations. Secondly, a significant

proportion of attendees had to be excluded because of missing or inadequate arterial stiffness and biomarker data. This is a well known but unavoidable limitation of large epidemiological studies that may bias toward the null hypothesis because of loss of cases that presumably had more extreme values for the analyzed variables. Thirdly, whereas the results are adjusted for multiple covariates that may be associated with homocysteine levels or with altered vascular properties, the possibility of residual confounding remains. For the last, the present study was performed in Chinese residents from two communities in Beijing; the results may not present Chinese from other area of China, and not be applicable to other ethnic groups.

In conclusion, a consistent positive relation of plasma homocysteine levels with CF-PWV was observed in men but not in women, indicating gender differences in the relations between homocysteine and large artery stiffness.

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