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ORIGINAL ARTICLE

Associations between plasma homocysteine levels, aortic stiffness and wave reflection in patients with arterial hypertension, isolated office hypertension and normotensive controls

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The aim of this study was to evaluate any possible association of homocysteine with arterial stiffness indices in patients with essential arterial hypertension (AH), isolated office hypertension (IOH) and normotensive controls. The final cohort comprised 231 normotensives (NTs, 119 males), 480 patients with IOH (196 males) and 1188 patients with essential AH (713 males). All patients were screened for plasma homocysteine levels and lipidaemic profile and underwent aortic compliance and wave reflection assessment by using carotid-femoral pulse wave velocity (PWVc-f) and aortic augmentation index corrected for heart rate (AIx) accordingly. In the total population, stepwise multiple linear regression analysis showed that homocysteine levels remained a significant determinant of PWV (beta (SE): 0.056 (0.007), $P < 0.001$) and AIx (beta (SE): 0.236 (0.052), $P < 0.001$) independently of the traditional factors affecting arterial stiffness and wave reflection. When the three groups were examined separately, homocysteine levels remained an independent determinant of PWFc-f in all groups (NT: beta (SE): 0.070 (0.022), $P = 0.002$; IOH: beta (SE): 0.109 (0.015), $P < 0.001$; AH: beta (SE): 0.040 (0.009), $P < 0.001$). However, homocysteine levels remained an independent determinant of AIx only in the IOH and AH, but not in the NT group (IOH: beta (SE): 0.302 (0.124), $P = 0.015$; AH: beta (SE): 0.183 (0.057), $P = 0.001$; NT: beta (SE): 0.308 (0.240), $P = 0.200$). This study points to an independent relationship between circulating homocysteine levels, aortic compliance and wave reflection.
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Keywords: homocysteine; arterial stiffness; wave reflection; isolated office hypertension

Introduction

Increased levels of plasma homocysteine have been considered as an independent risk factor for cardiovascular disease.[1,2] Specifically, high plasma homocysteine levels are related to increased risk for myocardial infarction, stroke, heart failure and occlusive peripheral arterial disease.[3–7] Furthermore, hyperhomocysteinaemia is associated with increased carotid intima-media thickness,[8] extracranial carotid artery stenosis[9] and more advanced atherosclerotic changes in coronary arteries.[10]

Among the possible mechanisms that these effects are exerted on are increased smooth muscle cell proliferation,[11] endothelial dysfunction,[12] increased collagen synthesis and deterioration of elastic material of the arterial wall.[13]

With regard to the relationship between homocysteine levels and hypertension, a synergistic effect of these two parameters on cardiovascular risk[10,14] and an independent relationship between high homocysteine levels and isolated systolic hypertension are reported,[15] which was attributed to arterial stiffening. Associations of homocysteine with cardiovascular risk and with alterations in vascular properties have led to studies of its association with arterial stiffness. Thus, there are reports of a significant association between plasma homocysteine pulse pressure[16] and arterial pulse wave velocity (PWV);[17,18] however, results are controversial as there are no reports of such associations.[19]

On account of this controversy in the earlier reports, the purpose of this study was to assess the relationship between plasma homocysteine levels, arterial stiffness and wave reflection indices in patients with arterial hypertension (AH), isolated office hypertension (IOH) and normotensive controls (NC).

Materials and methods

Study population

This retrospective study comprised 2150 patients who visited our outpatient clinic from 2005 to 2008. The study protocol has been approved by the Ethics Committee of Hippokration Hospital. Patients with acute and chronic inflammatory disease, endocrine disorders, chronic obstructive pulmonary disease, malignancy, renal insufficiency (serum creatinine $> 1.3 \, \text{mg} \, \text{dl}^{-1}$), heart failure, recent (< 6 months) cerebrovascular event, coronary artery disease, history of stable/unstable angina or past myocardial infraction, ventricular arrhythmia, sinus bradycardia (< 55 b.p.m.), sinus tachycardia (> 100 b.p.m.) or atrioventricular conduction defects, known diabetes mellitus (earlier antidiabetic treatment) and any condition preventing technically adequate ambulatory blood pressure (BP) monitoring were excluded from the study.

Thus, the final cohort comprised 1899 patients, of which 231 were normotensives (NT), 1188 presented with essential AH and 480 with IOH.

All participants were subjected to the following procedures: physical examination, medical history, repeated clinical BP measurement and blood sampling for laboratory examinations. Patients with borderline office BP and patients reporting normal BP levels outside the clinic were subjected to 24-h ambulatory BP monitoring.

BP measurements

The evaluation of BP was made according to the 2003 European Society of Hypertension/European Society of Cardiology guidelines for adults.[20] BP was measured three times with 1 min intervals and with the subject resting comfortably, back supported in the sitting position after a 10–15 min relax period. A mercury sphygmomanometer was used for all measurements with a medium- or a large-size cuff, according to the subject's arm circumference.

Furthermore, 40% of patients underwent ambulatory BP monitoring for 24 h on the non-dominant arm using a Spacelabs 90207 device (SpaceLabs Inc., Redmond, WA, USA). Technical details have been reported earlier.[21]

Anthropometric and biochemical measurements

For each subject, weight, height and waist to hip (W/H) ratio were measured, and body mass index was also calculated. Waist circumference was measured at the midpoint between the bottom of the rib cage and above the top of the iliac crest from patients at minimal respiration to the nearest 0.1 cm.

All subjects underwent full laboratory evaluation (lipidaemic profile, liver and kidney function indices). The blood samples were collected from the antecubital vein between 8 a.m. and 10 a.m., in a sitting position, after 12 h of fasting and alcohol absence. The biochemical evaluation was carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories.

Plasma total homocysteine levels were determined using the homocysteine microplate enzyme immunoassay assay (Bio-Rad Laboratories, Oslo, Norway)[22] (cut-off points: 4–25 µmol/l).

Measurements of arterial properties

Haemodynamic measurements were conducted in the morning, in a quiet environment, at stable temperature. Subjects were requested to abstain from caffeine, smoking and alcohol for at least 12 h before arterial properties assessment was performed. Systolic BP, diastolic BP and heart rate were measured twice in both arms with automatic mercury (Omron M4-I, CE 0197, Netherlands).

Aortic wave reflection assessment. We used a validated, commercially available system (SphygmoCor; AtCor Medical, Sydney, Australia), which uses the principle of applanation tonometry and appropriate acquisition and analysis software for non-invasive recording and analysis of the arterial pulse. First, from the radial artery recordings, the ensemble-averaged radial pressure waveform is obtained. Then, this is calibrated according to sphygmomanometric systolic and diastolic BP measured in the brachial artery (as there is practically negligible pressure pulse amplification between the brachial and the radial artery; subsequently, the mean radial pressure is calculated by digital integration of the waveform). Thereafter, from the radial artery pressure waveform, the *central* (aortic) arterial pressure waveform is derived.

Augmentation indices of the central waveform were measured as indices of wave reflection. *Augmentation index* was defined as augmented pressure divided by pulse pressure and expressed as a percentage. Augmentation index is a composite measure of the magnitude of wave reflection and arterial stiffness, which affects the timing of wave reflection.[23] Larger values of augmentation index indicate increased wave reflection from the periphery and/or earlier return of the reflected wave as a result of increased PWV (because of increased arterial stiffness), and vice versa. To take into account the potential effect of heart rate on augmentation index, all values were automatically corrected for heart rate by the sphygmocor software augmentation index corrected for heart rate (AIx).

Aortic stiffness assessment. The pulse travels at a higher velocity in a stiff aorta and vice versa. Carotid-femoral pulse wave velocity (PWVc-f) is a well-established index of arterial elastic properties and a prognosticator of cardiovascular risk. PWVc-f was calculated from measurements of pulse transit time and the distance travelled between two recording sites (PWV = distance (m)/transit time (s)) using a validated non-invasive device (Complior; Dupont Medical, Pantin, France), which allows online pulse wave recording and automatic calculation of PWV.[24] Two different pulse waves were obtained simultaneously at two sites (at the base of the neck for the common carotid and over the right-femoral artery) with two transducers. The distance was defined as (distance from the suprasternic notch to femoral artery)—(distance from the carotid artery to the suprasternic notch).

Statistical analysis. Data are presented as mean ± SEM except if differently indicated. Comparisons between the baseline characteristics of the three study groups (NC, IOH and AH) were performed after analysis of variance. Nominal data were compared by the χ^2-test. The determinants of PWV and augmentation index in the total population were identified by using Pearson's and Spearman's correlation coefficients for parametric and non-parametric variables accordingly. The independent determinants of PWVc-f and AIx in the total population were identified by using stepwise multiple linear regression analysis (dependent variables: PWVc-f or AIx; independent variables tested: age, gender, mean BP, body mass index, W/H, smoking status, total cholesterol, high-density lipoprotein, triglycerides, height, heart rate and homocysteine levels). The independent determinants of PWVc-f and AIx revealed by this analysis were used in two multiple linear regression models to assess the independent effect of the homocysteine levels on PWVc-f (model 1) and AIx (model 2) separately for each study group (NC, IOH and AH). Significance was assumed at a two-tailed probability value of <0.05.

Statistical analysis was performed using SPSS package for Windows version 13.0 (SPSS, Chicago, IL, USA).

Results

The baseline characteristics of the study population according to the office and ambulatory BPs are presented in Table 1. In the total population, both PWVc-f and AIx presented significant linear relations with age, male gender, smoking, peripheral systolic and diastolic BP, W/H ratio, smoking intensity assessed by pack years, total cholesterol, high-density lipoprotein and triglyceride levels (Table 2). PWVc-f was additionally correlated with body mass index and heart rate, which was not seen with AIx, whereas the latter was significantly associated with height (Table 2).

Finally, both PWVc-f and AIx were positively associated with the plasma homocysteine levels as shown in Table 2. In a stepwise multiple regression analysis, the plasma homocysteine levels remained a significant determinant of PWVc-f independent of

Table 1 Baseline characteristics of the study population according to the office and the ambulatory blood pressures

	Normotensives	Isolated office hypertension	Arterial hypertension	P
n (males)	231 (119)	480 (196)	1188 (713)	<0.001
Age (years)	45.8 ± 1.0	52.4 ± 0.6	52.4 ± 0.3	<0.001
Heart rate (bpm)	75.2 ± 0.4	74.2 ± 0.3	74.7 ± 0.2	0.104
Waist (cm)	88.1 ± 0.8	90.8 ± 0.6	95.8 ± 0.4	<0.001
Hip (cm)	103.1 ± 0.6	105.2 ± 0.4	106.9 ± 0.3	<0.001
Waist/hip	0.85 ± 0.005	0.86 ± 0.004	0.89 ± 0.002	<0.001
Body mass index (kg m^{-2})	26.2 ± 0.3	27.2 ± 0.2	28.2 ± 0.1	<0.001
Smokers (%)	37.7	36.7	41.2	0.192
Pack years	8.9 ± 1.0	9.5 ± 0.8	12.7 ± 0.6	0.001
Total cholesterol (mg dl^{-1})	199.5 ± 2.5	211.1 ± 1.7	210.7 ± 1.1	<0.001
High-density lipoprotein (mg dl^{-1})	52.2 ± 0.8	54.1 ± 0.6	50.1 ± 0.4	<0.001
Triglycerides (mg dl^{-1})	95.9 ± 2.9	109.1 ± 2.6	120.8 ± 1.7	<0.001
Peripheral systolic BP (mm Hg)	129.3 ± 0.5	157.6 ± 0.4	164.7 ± 0.3	<0.001
Peripheral diastolic BP (mm Hg)	82.0 ± 0.3	97.8 ± 0.3	100.9 ± 0.2	<0.001
Peripheral pulse pressure (mm Hg)	47.3 ± 0.4	59.8 ± 0.6	63.7 ± 0.4	<0.001
Mean BP (mm Hg)	97.8 ± 0.3	117.7 ± 0.2	122.2 ± 0.2	<0.001
Aortic systolic BP (mm Hg)	113.5 ± 0.8	127.2 ± 0.7	138.4 ± 0.5	<0.001
Aortic diastolic BP (mm Hg)	76.5 ± 0.6	83.6 ± 0.4	90.8 ± 0.3	<0.001
Aortic pulse pressure (mm Hg)	36.9 ± 0.6	43.6 ± 0.5	47.6 ± 0.4	<0.001
Aortic augmentation index (%)[a]	15.9 ± 1.0	23.3 ± 0.5	25.3 ± 0.3	<0.001
Reflection wave time delay (ms)	145.8 ± 1.2	138.9 ± 0.6	137.4 ± 0.3	<0.001
c-f pulse wave velocity (m s^{-1})	6.46 ± 0.08	7.23 ± 0.05	8.05 ± 0.04	<0.001
Homocysteine (μg dl^{-1})	9.3 ± 0.2	10.5 ± 0.2	11.7 ± 0.1	<0.001

Abbreviations: BP, blood pressure; c-f, carotid-femoral.
[a]Corrected for heart rate.

Table 2 Associations between demographic and haemodynamic variables with c-f pulse wave velocity and aortic augmentation index corrected for heart rate in the total population

| | c-f pulse wave velocity | | Aortic augmentation index[a] | |
	Correlation coefficient	P value	Correlation coefficient	P value
Age	0.425	<0.001	0.512	<0.001
Gender (male)[b]	0.064	0.005	−0.462	<0.001
Height	−0.027	0.241	−0.469	<0.001
Heart rate	−0.102	<0.001	−0.031	0.178
Peripheral systolic BP	0.446	<0.001	0.313	<0.001
Peripheral diastolic BP	0.127	<0.001	0.134	<0.001
Body mass index	0.169	<0.001	−0.041	0.071
Waist/hip	0.255	<0.001	−0.116	<0.001
Smoking[b]	0.063	0.006	0.148	<0.001
Pack years	0.152	<0.001	0.214	<0.001
Total cholesterol	0.148	<0.001	0.234	<0.001
High-density lipoprotein	−0.104	<0.001	0.198	<0.001
Triglycerides	0.160	<0.001	0.074	0.001
Homocysteine	0.333	<0.001	0.137	0.02

Abbreviations: BP, blood pressure; c-f, carotid-femoral.
[a]Corrected for heart rate.
[b]Spearman's correlation.

Table 3 Independent determinants of c-f pulse wave velocity and aortic augmentation index corrected for heart rate in the total population

| | c-f pulse wave velocity $R^2 = 0.319$ | | | | Aortic augmentation index[a] $R^2 = 0.508$ | | |
	beta	SE	P		beta	SE	P
Age	0.045	0.002	<0.001	Age	0.375	0.019	<0.001
Mean BP	0.036	0.003	<0.001	Mean BP	0.297	0.022	<0.001
Waist/hip	1.87	0.40	<0.001	Waist/hip	9.518	3.530	0.007
Homocysteine	0.056	0.007	<0.001	Homocysteine	0.236	0.052	<0.001
High-density lipoprotein	−0.009	0.003	0.001	Smoking	4.312	0.410	<0.001
				Males	−7.025	0.670	<0.001
				Height	−0.250	0.031	<0.001
				Body mass index	−0.293	0.051	<0.001
				Heart rate	0.138	0.035	<0.001
				Triglyceride	0.009	0.004	0.014

Abbreviations: BP, blood pressure; c-f, carotid-femoral.
[a]Corrected for heart rate.

the age, the mean BP, the W/H ratio and the high-density lipoprotein levels as shown in Table 3. In accordance, the stepwise multiple linear regression analysis also revealed an important association between homocysteine levels and AIx independent of other traditional factors affecting wave reflection, such as age, gender, smoking status, height, mean BP, body mass index, W/H ratio, heart rate and triglyceride levels (Table 3). These findings argue for a potential role of homocysteine in the remodelling of the arterial wall leading to arterial stiffness and enhanced wave reflection to the aorta.

When the three study groups were examined separately, homocysteine levels remained a significant independent determinant of PWVc-f in all groups as shown in Table 4. However, AIx was independently associated with the homocysteine levels in the groups of IOH and AH, but not in the NT group as shown in Table 4.

Discussion

The first finding of this study was that homocysteine is an independent determinant of large artery stiffness and wave reflections in the whole study cohort. This finding argues for a potential role of homocysteine in the remodelling of the arterial wall leading to arterial stiffness and enhanced wave reflection to the aorta. Earlier data suggest that homocysteine levels are associated with aortic stiffness in patients with type II diabetes mellitus.[25] In another study,[26] which involved health subjects, such an association between Hcy levels and arterial

Table 4 Independent effects of plasma homocysteine levels on carotid-femoral pulse wave velocity and aortic augmentation index corrected for heart rate separately for each study group according to the office and ambulatory blood pressures

	Normotensives beta (SE)	Isolated office hypertension beta (SE)	Arterial hypertension beta (SE)
c-f pulse wave velocity			
Homocysteine[a]	0.070 (0.022)	0.109 (0.015)	0.040 (0.009)
P	0.002	<0.001	<0.001
Aortic augmentation index[b]			
Homocysteine[c]	0.308 (0.240)	0.302 (0.124)	0.183 (0.057)
P	0.200	0.015	0.001

Abbreviation: c-f, carotid-femoral.
[a]After adjustment for age, mean blood pressure, waist/hip and HDL levels.
[b]Corrected for heart rate.
[c]After adjustment for age, mean blood pressure, waist/hip, smoking status, gender, height, body mass index, heart rate and triglyceride levels.

stiffness indices was not indicated, pointing to a clear controversy. The different results in the literature can be explained by the fact that hyperhomocysteinaemia may interact with other cardiovascular risk factors, namely, not only age and hypertension, but also smoking or diabetes mellitus.

The possible mechanisms of the relationship between Hcy and arterial stiffness are not yet fully well established. However, it is known that elevated Hcy levels induce oxidative injury to vascular endothelial cells and impair the production of nitric oxide, a strong relaxing factor by the endothelium.[27,28] Increased Hcy levels also enhance platelet adhesion to endothelial cells,[29] promote proliferation of vascular smooth muscle cells[30] and are associated with higher levels of prothrombotic factors, such as β-thromboglobulin, tissue plasminogen activator and factor VIIc.[31] In addition, oral folate supplementation is seen to improve the arterial endothelium-dependent vascular function of the brachial artery in healthy subjects with mildly elevated levels of Hcy.[32] Furthermore, patients with coronary artery disease and elevated Hcy, who were administered folate and vitamin B12, improved vascular endothelial function, as assessed by brachial artery flow-mediated dilatation.[33]

The second important finding of this study was that Hcy was an independent determinant of PWVc-f in healthy subjects as well as patients with isolate office hypertension and essential hypertension. It has been earlier analysed that serum Hcy levels enhance endothelial dysfunction. PWV is a known marker of arterial distensibility and endothelial function.[34] Although investigators have reported a significant association between plasma Hcy with pulse pressure[16] and arterial PWV in healthy subjects and patients with essential hypertension,[17] no such reports exist for patients with IOH. To our knowledge, this is the first study reporting such an association in white coat hypertensive patients. Patients with IOH are reported to present with impaired arterial distensibility, compared with NTs,

which entails a greater than normal cardiovascular risk.[35] According to this study, Hcy levels pose an additive burden to the cardiovascular profile of patients with IOH, leading to large artery stiffening independently of other factors.

Finally, this study revealed an independent relationship of Hcy with AIx in patients with essential hypertension and IOH, but not with NTs. AIx is a measure of the contribution made by the reflected pressure wave to the ascending aortic pressure waveform,[36] and thus provides a measure of systemic arterial stiffness.[37] Therefore, these data show that Hcy levels are associated with systemic arterial stiffness. The fact that this relation was not noted in NT subjects may be explained by the fact that AIx is a marker mainly of small artery stiffness, and thus it can be assumed that Hcy does not affect the arterial wall of small arteries in subjects with the lack of additional risk factors. Furthermore, a number of factors such as gender, heart rate, height, smoking and lipidaemic profile are known to affect AIx values,[38,39] which was also confirmed by the present study results. It is possible that these factors affect small artery stiffness to a greater extent in NT patients than does Hcy. Finally, the small number of participants in the NTs group may have affected the results by underestimating the effect of Hcy on AIx.

There are some limitations in this study. First, patients with masked hypertension were not included, which could have led to interesting comparisons. Second, AIx measurement was made using the methodology from reconstructed waveforms using a transfer function, which has been criticized and may, therefore, not detect changes in stiffness in the small arteries. However, this is a large study cohort that leads to strong and valid conclusions.

In conclusion, in hypertensive patients, patients with IOH and NT subjects, there is a strong and positive correlation of increased Hcy levels with PWV. These results suggest that the evaluation of aortic distensibility and Hcy levels can help in cardiovascular risk assessment both in hypertensive

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subjects and patients with IOH as well as in NT subjects. The contribution of arterial stiffness and plasma Hcy measurements in risk assessment and risk reduction strategies in such populations needs to be confirmed by large prospective interventional and observational studies.

What is known about this topic
- Increased levels of plasma Hcy are considered an independent risk factor for cardiovascular disease.[1,2]
- A significant association between plasma Hcy, pulse pressure and pulse wave velocity has been reported.[16–18]
- However, these results are controversial.[19,26]

What this study adds
- In hypertensive patients, in patients with IOH and normotensive subjects, there is a strong and positive correlation of increased Hcy levels with PWV.
- Hcy is an independent determinant of systemic arterial stiffness, as expressed through AIx, is essential hypertensives and in patients with IOH.
- Thus, the evaluation of aortic distensibility and Hcy levels can help in cardiovascular risk assessment both in hypertensive and patients with IOH as well as in normotensive subjects.

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