# Plasma Homocysteine, Aortic Stiffness, and Renal Function in Hypertensive Patients

Luiz A. Bortolotto, Michel E. Safar, Eliane Billaud, Christian Lacroix, Roland Asmar, Gérard M. London, Jacques Blacher

*Abstract*—Hyperhomocysteinemia has been associated with both vascular structure alterations and vascular clinical end points. To assess the relation between plasma homocysteine, structure and function of large arteries, and the presence of clinical vascular disease, we investigated a population of 236 hypertensive patients. We estimated arterial stiffness by measuring the carotid-femoral pulse wave velocity. Total plasma homocysteine was determined by fluorometric high-performance liquid chromatography. The presence of cardiovascular disease was defined on the basis of clinical events, including coronary heart disease, cerebrovascular disease, and peripheral vascular disease. In this population, pulse wave velocity was positively correlated with homocysteine, even after adjustments for age, mean blood pressure, extent of atherosclerosis, and creatinine clearance ($P=0.016$). Analysis of variance showed statistically significant differences between the mean values of homocysteine, creatinine clearance, and pulse wave velocity according to the extent of atherosclerosis, with an increase in these 3 parameters concomitant with an increase in the number of vascular sites involved with atherosclerosis. In conclusion, in hypertensive patients the levels of homocysteine are strongly and independently correlated to arterial stiffness measured by aortic pulse wave velocity. Plasma homocysteine, creatinine clearance, and aortic pulse wave velocity are higher in patients presenting with clinical vascular disease. These results suggest that the evaluation of aortic distensibility and homocysteine levels can help in cardiovascular risk assessment in hypertensive populations. (***Hypertension*. 1999;34[part 2]:837-842.)**

**Key Words:** homocysteine ■ arteries ■ blood flow velocity ■ vascular diseases ■ hypertension, renovascular

Cardiovascular (CV) disease (CVD) is a major cause of morbidity, dependence, and mortality worldwide. Besides the well-accepted CV risk factors such as smoking, diabetes mellitus, dyslipidemia, and hypertension, hyperhomocysteinemia is emerging as an independent and graded risk factor for stroke, myocardial infarction, and CV death.[1–3] Although there is considerable epidemiological evidence for a relationship between plasma homocysteine and CVD, not all prospective studies have supported such a relationship.[4–8] Moreover, it is not known whether a reduction in plasma homocysteine will reduce CVD risk.[9,10] Experimental studies have demonstrated that hyperhomocysteinemia can induce smooth muscle cell proliferation,[11] endothelial dysfunction,[12] collagen synthesis, and deterioration of elastic material of the arterial wall.[13] In humans, plasma homocysteine levels were found to be positively correlated with carotid artery intimal-medial wall thickness[14] and with extracranial carotid artery stenosis.[15]

Regarding the relationships between homocysteine and hypertension, 2 studies noted a synergistic effect of these parameters on CV risk,[16,17] and Sutton-Tyrrell et al[18] reported an independent relationship between high homocysteine levels and isolated systolic hypertension in older adults; the authors hypothesized a causal relationship between hyperhomocysteinemia and isolated systolic hypertension through arterial stiffening. We recently reported, in patients with end-stage renal disease (ESRD), a strong and independent association between homocysteine levels and lower-limb pulse wave velocity (PWV), a standard marker of arterial stiffness.[19] Because renal function is a strong determinant of both plasma homocysteine levels[20] and CV risk,[21] homocysteine-CVD relationships should be adjusted on this possibly confounding parameter.

In the present study, we investigated a large population of hypertensive patients with or without the presence of clinical CVD by determining the aortic PWV in conjunction with several clinical and biochemical parameters related to atherosclerosis, with an emphasis on renal function and homocysteine. The aim was to assess the relationships between arterial stiffness, renal function, and plasma homocysteine levels in conjunction with atherosclerosis.

## Methods

### Population

The study population consisted of 236 consecutive hypertensive patients (147 men and 89 women, with a mean±SD age of 58±13

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From the Department of Internal Medicine and Inserm U 337, Broussais Hospital, APHP, Paris, France.

Correspondence to Pr M. SAFAR, Hôpital Broussais, Service de Médecine Interne, 96, rue Didot, 75014 Paris, France. E-mail michel.safar@brs.ap-hop-paris.fr



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**TABLE 1. Characteristics of Patients According to the Presence or Absence of Clinical Vascular Disease**

| Parameter | Clinical Vascular Disease (n=40) | No Clinical Vascular Disease (n=196) | *P* |
|---|---|---|---|
| Age, y | 62±13 | 57±13 | 0.03 |
| Sex, M/F | 29/11 | 118/78 | ... |
| SBP, mm Hg | 148±21 | 144±21 | ... |
| DBP, mm Hg | 83±13 | 83±12 | ... |
| MBP, mm Hg | 105±14 | 103±14 | ... |
| Pulse pressure, mm Hg | 65±18 | 61±17 | ... |
| Heart rate, bpm | 65±10 | 66±10 | ... |
| Current smoker, % | 25 | 19 | ... |
| Tobacco lifelong dose, pack-y | 25±28 | 10±16 | <0.0001 |
| Duration of anti-HTA therapy, y | 12±9 | 8±9 | 0.04 |
| Body mass index, kg/m$^2$ | 26±3 | 27±5 | 0.05 |
| Waist-to-hip ratio | 0.96±0.08 | 0.95±0.08 | ... |
| Total cholesterol, mmol/L | 5.4±1.0 | 5.6±1.1 | ... |
| LDL cholesterol, mmol/L | 3.6±0.9 | 3.7±1.0 | ... |
| Plasma glucose, mmol/L | 5.8±1.0 | 6.0±1.4 | ... |
| Plasma creatinine, μmol/L | 115±51 | 89±25 | <0.0001 |
| Creatinine clearance, mL/min | 69±30 | 90±34 | 0.0002 |
| Plasma homocysteine, μmol/L | 15.9±5.3 | 13.6±4.9 | 0.007 |
| PWV, m/s | 14.3±4.0 | 12.4±2.7 | 0.0003 |

HTA indicates hypertension. Continuous variables are expressed as mean±SD.

years) who entered the Broussais Hospital Internal Medicine Department in 1997 for a CV checkup because of the presence of CV risk factors associated with high blood pressure (BP): smoking, dyslipidemia, diabetes mellitus, and/or a family history of premature CVD, with or without previous CVD. The diagnosis of hypertension was established on the basis of a systolic BP (SBP) >140 mm Hg and/or a diastolic BP (DBP) >90 mm Hg measured by mercury sphygmomanometry in the supine position, with a minimum of 3 casual measurements during the last month in never-treated hypertensive subjects (n=34) and by the presence of antihypertensive treatment (n=202), regardless of whether or not BP was well controlled (SBP<140 mm Hg and DBP<90 mm Hg). Patients with all forms of secondary hypertension (on the basis of standard laboratory and radiology tests), cancer (other than basal cell carcinoma), insulin-dependent diabetes mellitus, or severe renal insufficiency (creatinine>300 μmol/L) or those taking vitamin B supplements were not included in the study. Among the 202 patients who were being treated with antihypertensive therapy at the time of inclusion into the study, the mean number of antihypertensive drugs was 1.8±0. per patient. The antihypertensive drugs included calcium antagonists (115 patients), β-blockers (80 patients), angiotensin-converting enzyme inhibitors (66 patients), diuretics (63 patients), central-acting agents (26 patients), angiotensin II antagonists (11 patients), and α-blockers (1 patient), either alone or in combination. Forty-two patients (18%) were being treated for dyslipidemia and 24 patients (10%) for diabetes mellitus. Each subject provided informed consent for the study, which was approved by our institutional review board.

The following information was obtained from a questionnaire filled out at the time inclusion into the study: sex; age; weight; height; personal history of diabetes mellitus, dyslipidemia, and smoking; previous diseases; and use of current medications. From this questionnaire and the complementary findings during hospitalization, CVD was present in 40 patients and absent in 196 patients. The definition of CVD was based on the usual criteria according to the International Classification of Diseases (ninth revision) for coronary heart disease (CHD), cerebrovascular disease, and peripheral vascular disease.

The CVD involved at least 1 vascular site, including CHD (24 patients), cerebrovascular disease (20 patients), and peripheral vascular disease (14 patients). The mean number of vascular sites involved with CVD in the population of the 40 patients was 1.45±0.60 per patient. Extent of atherosclerosis was assessed as the number of vascular sites involved with CVD: 0 (196 patients), 1 (24 patients), 2 (14 patients), or 3 (2 patients).

## Procedures

The measurements were performed in the morning after an overnight fast, each patient being in the supine position. Brachial BP was measured using a mercury sphygmomanometer after 15 minutes of rest. Phases I and V of the Korotkoff sounds were considered respectively as SBP and DBP. The mean BP (MBP) was calculated as DBP+(SBP−DBP/3). Five measurements taken 2 minutes apart were averaged.

After BP determination, the PWV measurement was performed, before 3-lead orthogonal electrocardiography and blood sampling were done, in a controlled environment at 22±2°C. PWV was determined using an automatic device, the Complior (Colson, France), which allows an online pulse wave recording and automatic calculation of PWV.[22] In brief, common carotid artery and femoral artery pressure waveforms were recorded noninvasively using a TY-306 Fukuda pressure-sensitive transducer (Fukuda, Tokyo, Japan). The pressure waveforms were digitized at the sample acquisition frequency of 500 Hz. The 2 pressure waveforms were then stored in a memory buffer. A preprocessing system automatically analyzed the gain in each waveform and adjusted it for equality of the 2 signals. Details of this procedure have been previously published.[22] When the operator observed a pulse waveform of sufficient quality on the computer screen, digitization was suspended and calculation of the time delay between the 2 pressure upstrokes was initiated. Measurement was repeated over at least 10 different cardiac cycles, and the mean was used for the final analysis. The distance traveled by the pulse wave was measured over the body surface as the distance between the 2 recording sites (*D*), while pulse transit time (*t*), measured between the feet of the pressure waveforms

recorded at these different points (foot-to-foot method), was automatically determined by the Complior; PWV was automatically calculated as $PWV=D/t$. The validation of this automatic method and its reproducibility have been previously described, with an intraobserver repeatability coefficient of 0.935 and an interobserver reproducibility coefficient of 0.890.[22]

On the basis of the 8-second, 3-lead orthogonal electrocardiographic recording, the average heart rate was calculated (in beats per minute). Waist circumference midway between the lowest rib and iliac crest and the hip circumference at the level of the great trochanters were measured with flexible tape. Venous blood samples were obtained from subjects after an overnight fast. Plasma was separated without delay at 4°C in a refrigerated centrifuge and stored at 4°C (for the determination of routine chemistry profile by standard methods) until analysis. Creatinine clearance was calculated according to the Cockcroft and Gault formula.[23] Total cholesterol and triglycerides were determined using a Technicon Chem assay (Technicon Instruments, Tarrytown, NY), and HDL cholesterol was measured in the supernatant after precipitation of apolipoprotein B–containing lipoproteins with heparin-$MnCl_2$. LDL cholesterol was calculated by the formula of Friedewald et al[24] for patients with serum triglyceride concentrations <4.0 mmol/L.

Total homocysteine, the sum of the acid-soluble (ie, reduced homocysteine, homocystine disulfide, and homocysteine-cystine mixed disulfide) and protein-bound moieties, was determined in plasma by the fluorometric high-performance liquid chromatography method originally described by Fortin and Genest.[25] The interassay and intra-assay coefficients of variation for this assay were both <8%. Normal values for plasma total homocysteine are <16 μmol/L.[25]

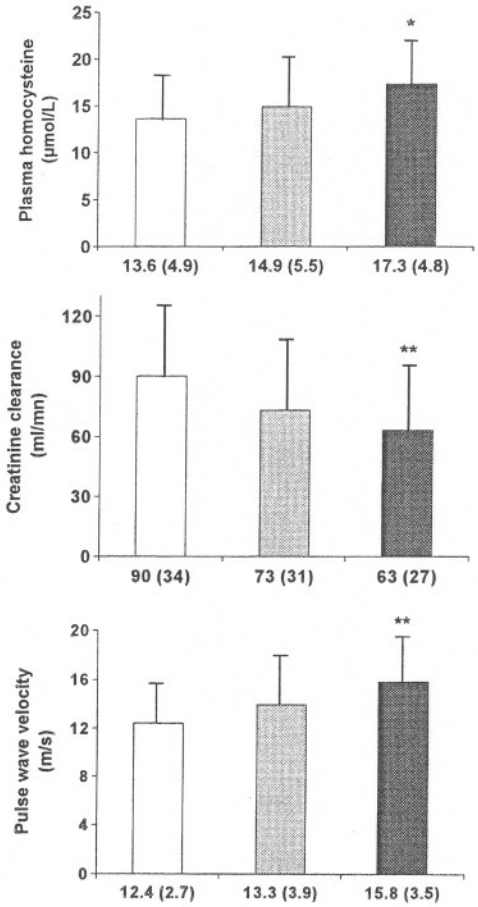
## Statistical Analysis

Data were expressed as mean±SD. A Student's $t$ test was used for comparison of normally distributed continuous variables. Differences in frequency were tested by $\chi^2$ analysis. Sex was used as a dummy variable (1=male, 2=female). Robust multiple regression analysis was performed to assess linear correlations between aortic PWV, determinants of clinical and biochemical parameters, and their interactions. ANOVA was used to test the differences of the mean values of aortic PWV, creatinine clearance, and plasma homocysteine, according to the number of vascular sites involved with atherosclerosis. Patients with 3 sites (2 patients) and patients with 2 sites (14 patients) were analyzed within the same group because of small numbers. Statistical analysis was performed with NCSS 6.0.21 software.[26] A $P$ value <0.05 was considered significant. All testing was 2-sided.

## Results

In our study population, we noted a positive correlation between plasma homocysteine and age ($r=0.37$, $P<0.0001$) and between plasma homocysteine and plasma creatinine ($r=0.53$, $P<0.0001$). Plasma homocysteine was negatively correlated with calculated creatinine clearance ($r=-0.48$, $P<0.0001$). Although plasma homocysteine was higher in men than in women (14.4±4.9 versus 13.2±5.2 μmol/L), this difference did not reach statistical significance ($P=0.08$).

Table 1 shows the characteristics of the patients according to the presence or absence of CVD. Mean±SD plasma homocysteine was 15.9±5.3 μmol/L in the group of patients with CVD and 13.6±4.9 μmol/L for the patients without CVD ($P=0.007$). Mean±SD PWV was 14.3±4.0 m/s in the group of patients with CVD and 12.4±2.7 m/s for patients without CVD ($P=0.0003$). Patients with CVD were older ($P=0.03$), had higher plasma creatinine ($P<0.0001$) and lower creatinine clearance ($P=0.0002$), had been treated longer for hypertension ($P=0.04$), had a higher tobacco lifelong dose ($P<0.0001$), and presented a lower body mass index ($P=0.05$).




Plasma homocysteine, creatinine clearance, and carotid-femoral PWV according to the extent of atherosclerosis. Differences in the mean values were assessed by 1-way ANOVA.

In the Figure, we present the levels of plasma homocysteine, creatinine clearance, and aortic PWV according to the presence and extent of CVD. ANOVA showed statistically significant differences between the mean values, with an increase of these 3 parameters with an increase in the number of vascular sites involved with atherosclerosis.

We found a positive correlation, in univariate analysis, between plasma homocysteine and aortic PWV ($r=0.44$, $P<0.0001$, data not shown). Considering aortic PWV as a dependent variable, robust multivariate regression analysis showed that the only parameters entering the model were age ($P<0.0001$), MBP ($P<0.0001$), extent of atherosclerosis ($P<0.0001$), plasma homocysteine ($P=0.005$), and to a lesser extent, creatinine clearance ($P=0.09$, Table 2).

## Discussion

The salient finding of our study is that in hypertensive patients, plasma homocysteine levels were strongly correlated

**TABLE 2. Robust Multiple Regression Analysis of Aortic Pulse Wave Velocity (PWV)**

| Parameters | Regression Coefficient | SE | *t* Value | *P* |
|---|---|---|---|---|
| Age, y | 0.095 | 0.009 | 10.9 | <0.0001 |
| Mean arterial pressure, mm Hg | 0.048 | 0.007 | 7.2 | <0.0001 |
| Extent of atherosclerosis, (0, 1, or 2) | 0.934 | 0.171 | 5.4 | <0.0001 |
| Plasma homocysteine, μmol/L | 0.067 | 0.021 | 3.2 | 0.005 |
| Creatinine clearance, mL/min | 0.006 | 0.004 | 1.7 | 0.09 |

Dependent variable: aortic PWV, m/s ($r^2$=0.58, F ratio=59.7, $P$<0.0001).

with aortic PWV, independent of age, BP, extent of atherosclerosis, and renal function. In the present study, we used PWV as a marker of arterial stiffness. PWV measurement offers a simple, reproducible, indirect, and noninvasive evaluation of regional arterial stiffness.[22,27–30] The PWV determined from foot-to-foot transit time in the aorta eliminates the influence of wave reflections and is close to the characteristic PWV determined from phase velocities.[27] The critical factors are the precise measurements of this transit time and the length of the vascular segments. Transcutaneous determination of vessel length is an approximation that might underestimate vascular length, an error that might arise especially in elderly patients with an unfolded, tortuous aorta. Despite these limitations, measurement of PWV is strongly correlated with direct measurements of arterial distensibility and can be considered a good surrogate for the evaluation of arterial stiffness by phase-locked echo-tracking systems.[31] Aortic PWV increases with age and BP pressure and must be interpreted accordingly. Studies in ESRD patients have shown that arterial stiffness is enhanced independently of age and BP.[31] Relationships between arterial stiffness and CV risk should then be adjusted on age, BP, and also renal function.

In the present study, the strong relationship between homocysteine and renal function is not surprising. Plasma homocysteine is increased in patients with ESRD,[32] which is related to several mechanisms involving, in addition to the loss of urinary excretion, the possible defect in vitamin $B_6$–dependent transsulfuration of homocysteine,[33] the decreased extrarenal catabolism in this disease state,[34] and the loss of considerable metabolism of homocysteine by normal renal parenchyma.[35] This latter factor seems to be the most important determinant of the refractory hyperhomocysteinemia observed in ESRD,[36] since daily renal excretion of homocysteine is just 0.1% of total daily production.[37] Indeed, we have recently demonstrated in patients with ESRD that lower-limb but not aortic PWV is strongly correlated with homocysteine levels.[19] In this study, plasma homocysteine levels did not differ in the presence or absence of native kidneys, indicating that the altered renal parenchyma did not contribute substantially to the increased plasma homocysteine levels. Moreover, the correlation of homocysteine and PWV was independent of creatinine clearance in this population with "normal" renal function. The different results in the literature can be explained by the fact that hyperhomocysteinemia may interact with other CV risk factors, namely, not only age, hypertension, and renal insufficiency[20] but also smoking or non–insulin-dependent diabetes mellitus.[1,2,9,10] In a study on elderly subjects, homocysteine was associated with isolated systolic hypertension and was also related to atherosclerosis, but only in normotensive individuals,[18] and in patients with non—insulin-dependent diabetes mellitus, plasma homocysteine levels were associated with DBP and MBP.[38]

Another important result of our study is that both PWV and homocysteine levels were found to be significantly elevated in the presence of CVD; moreover, the highest values were observed when atherosclerotic disease was present in 2 or 3 vascular sites. The association between homocysteine levels and extent of atherosclerosis as assessed by angiography or ultrasound yields conflicting results.[3,14–16,37,39,40] Recently, the Homocysteine and Progression of Atherosclerosis Study,[41] a prospective study on the influence of homocysteine levels on atherosclerosis progression in patients with symptomatic peripheral arterial disease, showed a strong association of elevated plasma homocysteine with the progression of CHD and death. In the present study, in addition to the choice of an arterial parameter (PWV) exploring both structural and functional properties combined, we wished to explore substantially more of the arterial tree than a carotid or femoral segment. We then chose the whole aorta. These elements can partly explain the difference between our results and those from Smilde et al,[39] which showed only a marginal influence of homocysteine on arterial stiffness at the sites of carotid and femoral arteries, probably because the biochemical and/or structural factors related to atherosclerosis may differ according to the topography of arterial vessels and to the extent of atherosclerosis.

Plausible biological mechanisms have been proposed by which high plasma homocysteine levels may lead to vascular damage: homocysteine may induce endothelial dysfunction manifested as impaired endothelium-dependent vasodilatation,[12] and they could stimulate vascular smooth muscle cell proliferation.[11] In a recent experimental study in minipigs,[13] the authors reported that mild hyperhomocysteinemia cause an arterial site–dependent deterioration of the elastic structure involving metalloproteinase-related elastolysis. Because the composition of arterial wall material, namely, smooth muscle cells and extracellular matrix, is a strong determinant of PWV, the present positive correlation between PWV, extent of atherosclerosis, and homocysteine points to the presence of diffuse and calcified atherosclerotic plaques in association with the development of extracellular matrix, mainly collagenous tissue.

The most important limitation of the present study involves the patient population. We studied a consecutive series of hospital-referred patients, which does not constitute a valid epidemiological population sample. Such a population is heterogeneous in nature, including never-treated hypertensives and patients on treatment with a wide range of single- and multiple-drug therapies with a variable degree of BP control, and also including patients on lipid-lowering drugs. Moreover, it should be noted that although a significant proportion of patients (17%) had confirmed CVD, this proportion was probably underestimated, including unrecognized silent myocardial ischemia or cerebrovascular disease, since invasive explorations were not systematically performed. From a methodological point of view, therefore, the relation between homocysteine, PWV, atherosclerosis, and renal function cannot be directly extended to other populations. The ability to generalize the results of the present study may also be limited because of renal function evaluation. Although the Cockcroft and Gault formula is a well-accepted creatinine clearance evaluation,[23] this calculation probably does not result in a very sensitive detection of minor renal dysfunction. Bostom and colleagues[42] have shown that serum cystatin C is a more sensitive measure of renal function, at least in terms of its impact on plasma homocysteine. Thus, the apparent independent association between homocysteine and arterial stiffness could be confounded by minor degrees of renal dysfunction, undetected by calculated creatinine clearance.

In conclusion, in hypertensive patients the levels of homocysteine are strongly correlated to arterial stiffness measured by aortic PWV. Of course, given that our study is cross sectional, we have no direct evidence for a cause-effect interaction. Also, both aortic PWV and homocysteine are higher in patients presenting with CVD. These results suggest that the evaluation of aortic distensibility and of homocysteine levels can help in CV risk assessment in hypertensive populations. The contribution of arterial stiffness and plasma homocysteine measurements in risk assessment and risk reduction strategies in hypertensive populations needs to be confirmed by large observational and interventional prospective studies.

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