Higher Plasma Homocysteine Concentration Is Associated with More Advanced Systemic Arterial Stiffness and Greater Blood Pressure Response to Stress in Hypertensive Patients

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Hyperhomocysteinemia has been reported to be associated with both vascular structure alteration and increased cardiovascular risk. This study examined whether hyperhomocysteinemia causes increased systemic arterial stiffness, thereby enhancing blood pressure response to stress in hypertensive patients. In 50 treated hypertensive patients, we studied brachial-ankle pulse wave velocity (PWV), a new measure for arterial stiffness, blood pressure response to stress, and blood pressure recovery after stress. Autonomic nervous activities were examined by spectral analysis of blood pressure and RR interval variabilities. Total plasma homocysteine and neurohumoral parameters were determined from fasting blood. Brachial-ankle PWV correlated with age ($r=0.64$, $p<0.001$), plasma homocysteine concentration ($r=0.35$, $p<0.05$), and systolic blood pressure (SBP) ($r=0.62$, $p<0.001$). Higher plasma homocysteine concentration was independently associated with greater brachial-ankle PWV ($\beta=0.388$, $p=0.01$). We classified the subjects into high homocysteine (7.3 nmol/ml or over) and low homocysteine (7.2 nmol/ml or below) groups. Baseline SBP, plasma renin activity, aldosterone, and norepinephrine concentrations were similar between the two groups. However, the SBP values during stress and the recovery periods were higher in the high homocysteine group than the low homocysteine group even after adjusting for sex and age. The behavior of sympathetic vasomotor activity did not differ between the two groups. These data suggest that higher plasma homocysteine concentration is associated with increased systemic arterial stiffness, which may enhance blood pressure reactivity to stress in hypertensive patients. (*Hypertens Res* 2006; 29: 403–409)

Key Words: homocysteine, arterial stiffness, autonomic nervous system, blood pressure, stress

Introduction

Increased level of plasma homocysteine has been well accepted as an independent risk factor for cardiovascular diseases (1–3). In humans, it has been shown that a high level of plasma homocysteine is associated with increased carotid intima-media thickness (4) and more advanced atherosclerotic changes in coronary arteries (5). Experimental studies

have demonstrated that hyperhomocysteinemia can induce smooth muscle cell proliferation (6), endothelial dysfunction (7), collagen synthesis, and deterioration of elastic material of the arterial wall (8). Bortolatto *et al.* have shown that plasma homocysteine concentration correlated positively with aortic stiffness as assessed by carotid-femoral pulse wave velocity in hypertensive humans (9). Thus plasma homocysteine could exert direct atherosclerotic effects on the arterial wall.

In addition, homocysteine may cause atherosclerotic arte-

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rial changes by elevating blood pressure. Homocysteine has been reported to play a role in insulin resistance (10). Insulin resistance could increase sympathetic nervous activity and Na reabsorption in the proximal tubules of the kidney, thereby increasing blood pressure (11). In fact, previous studies have shown that plasma homocysteine concentration is correlated with systolic blood pressure in humans (12, 13).

A series of events such as atherosclerotic changes in the arterial wall, Na retention, and sympathetic activation may promote blood pressure response to stress. Greater blood pressure reactivity has been reported to be associated with more advanced carotid atherosclerosis in post-menopausal women (14). We have shown that stress-related increase in blood pressure is an independent contributor to left ventricular hypertrophy in hypertensive men (15). Moreover, a recent study has shown that impaired blood pressure recovery after stress could predict future morbidity of hypertension in normotensive subjects (16). Therefore, the factors that regulate blood pressure reactivity to stress are of clinical importance. It remains unclear, however, whether plasma homocysteine modulates blood pressure response to stress. The aim of this study was to examine whether high plasma homocysteine concentration is associated with increased arterial stiffness and blood pressure reactivity to stress in hypertensive patients.

Methods

Subjects

We evaluated 50 patients (23 male and 27 female, mean age 56.1 ± 13.8 years, range 21–78 years) with essential hypertension but without severe organ damage (Table 1). All patients were on antihypertensive medications that they stopped at least 24 h before the study. The experiments were performed between 9:00 AM and 11:00 AM. All subjects were instructed to avoid cigarettes and beverages containing caffeine starting from the previous evening. Patients with secondary hypertension, cancer, insulin dependent diabetes mellitus, and renal insufficiency (plasma creatinine concentration >1.5 mg/dl) were not included in the study. The antihypertensive drugs included calcium channel blockers (12 patients [24.0%]), β -blockers (8 patients [16.0%]), angiotensin converting enzyme inhibitors (1 patient [2.0%]), diuretics (7 patients [14.0%]), and angiotensin II receptor antagonists (4 patients [8.0%]). The mean number of antihypertensive drugs was 1.2 per patient. Twelve patients (24.0%) were being treated for dyslipidemia and one (2.0%) for diabetes mellitus. The study protocol was approved by the Ethics Committee of Tohoku Rosai Hospital and informed consent was obtained from all subjects.

Procedures

The measurements were performed in the morning after an

Table 1. Demographic and Clinical Characteristics of Subjects

Variables	All (N=50)	Minimum	Maximum
Age (years)	56.1 ± 13.8	21	78
Body mass index (kg/m ²)	26.1 ± 4.9	19.0	38.9
Casual SBP (mmHg)	144 ± 10	130	164
Casual DBP (mmHg)	90 ± 9	69	109
Brachial SBP (mmHg)	141 ± 17	107	181
Brachial DBP (mmHg)	86 ± 10	70	117
Heart rate (beats/min)	67 ± 10	50	87
Pulse wave velocity (cm/s)	$1,540 \pm 338$	933	2,460
Homocysteine (nmol/ml)	7.5 ± 2.1	3.2	12.7
Creatinine (mg/dl)	0.7 ± 0.2	0.4	1.5
Insulin (μ U/ml)	7.6 ± 5.5	1.0	28.5
Renin (ng/ml/h)	1.1 ± 1.5	0.1	8.3
Aldosterone (pg/ml)	70 ± 31	23	160
Epinephrine (pg/ml)	36 ± 27	5	109
Norepinephrine (pg/ml)	354 ± 130	63	603
Fasting blood sugar (mg/dl)	104 ± 23	75	181
HbA1c (%)	5.5 ± 0.9	4.7	9.4
Total cholesterol (mg/dl)	192 ± 30	125	269
HDL cholesterol (mg/dl)	56 ± 14	38	90
Triglycerides (mg/dl)	109 ± 71	34	335

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

overnight fast with the patient in a supine position. A catheter was inserted into the left antecubital vein for blood sampling. Blood was drawn after 30 min of rest. Metabolic parameters such as fasting blood sugar, HbA1c, total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations were measured by standard methods. Plasma renin activity and plasma aldosterone concentration were measured by radioimmunoassay (RIA) using commercially available RIA kits (SRL, Tokyo, Japan; and Daiichi, Tokyo, Japan). Plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography (TOSOH, Tokyo, Japan). Plasma insulin concentration was measured by RIA using a commercially available kit (Dinabot, Tokyo, Japan). Total homocysteine concentration was determined by high performance liquid chromatography (LC-9A; Shimadzu, Kyoto, Japan).

Systemic arterial stiffness was then studied by means of brachial-ankle pulse wave velocity (PWV). Details of the methodology and clinical application of this measure have been previously described (17, 18). In brief, the instrument simultaneously records right and left brachial and tibial arterial pressure wave forms, lead I of the ECG, and a phonocardiogram. Occlusion cuffs, which were connected to both plethysmographic and oscillometric sensors, were placed around both arms and ankles for pulse wave analysis and blood pressure measurements. The time difference between

Table 2. Correlation Matrix of Homocysteine and Related Variables in All Subjects

	Homocysteine	Age	BMI	SBP	DBP	HR	PWV	Creatinine	Insulin	Renin	Aldo	E	NE	FBS	HbA1c	T-cho	HDL-cho
Age	0.16																
BMI	-0.03	-0.29															
SBP	0.27	0.35*	-0.02														
DBP	0.37*	0.07	0.02	0.71***													
HR	-0.04	0.02	0.19	0.29	0.32												
PWV	0.35*	0.64***	-0.29	0.62***	0.36*	0.41*											
Creatinine	0.66***	0.43*	-0.16	0.28	0.27	0.18	0.33										
Insulin	-0.07	-0.13	0.62***	-0.02	-0.03	0.45*	-0.11	-0.09									
Renin	0.29	-0.09	0.00	-0.10	0.17	0.43*	0.18	0.01	0.02								
Aldo	0.10	-0.35*	0.33	-0.07	0.28	0.33	0.01	0.07	0.40**	0.44**							
E	0.02	-0.23	-0.33	-0.11	0.07	0.02	-0.09	0.12	-0.15	0.02	0.10						
NE	0.13	0.12	-0.03	0.28	0.12	0.40*	0.22	0.01	-0.19	0.10	-0.14	0.16					
FBS	-0.24	0.08	0.14	0.19	0.11	-0.05	0.15	-0.02	0.55***	-0.12	0.22	-0.15	-0.10				
HbA1c	-0.13	-0.01	0.43**	0.09	0.15	0.36*	-0.08	0.14	0.63***	-0.12	0.23	-0.26	-0.05	0.75***			
T-cho	-0.35*	-0.13	0.17	-0.14	-0.13	0.08	-0.10	-0.53*	0.16	-0.04	0.10	-0.12	0.11	0.41**	0.20		
HDL-cho	-0.08	0.15	-0.38*	0.09	-0.09	-0.14	0.26	-0.37*	-0.37*	-0.11	-0.27	0.09	0.28	0.03	-0.21	0.19	
Triglycerides	-0.04	-0.12	0.10	-0.12	-0.04	0.02	-0.12	0.05	0.38*	0.25	0.10	-0.14	-0.22	0.45**	0.39*	0.40**	-0.23

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; PWV, pulse wave velocity; Aldo, aldosterone; E, epinephrine; NE, norepinephrine; FBS, fasting blood sugar; T-cho, total cholesterol; HDL-cho, high-density lipoprotein cholesterol. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3. Demographic Data in the High and Low Homocysteine Groups

	High homocysteine group (N=25)	Low homocysteine group (N=25)	<i>p</i> value
Homocysteine (nmol/ml)	9.0±1.8	6.0±1.0	0.0001
Age (years)	52.1±15.9	58.6±11.1	n.s.
Sex (male, %)	72.2	16.7	0.003
Body mass index (kg/m ²)	25.1±4.8	26.8±6.1	n.s.
Casual SBP (mmHg)	143±8	148±12	n.s.
Casual DBP (mmHg)	94±10	88±9	n.s.
Brachial SBP (mmHg)	143±17	141±14	n.s.
Brachial DBP (mmHg)	91±12	85±5	n.s.
Heart rate (beats/min)	66±10	71±8	n.s.
Pulse pressure (mmHg)	52.0±9.6	55.8±10.1	n.s.
Pulse wave velocity (cm/s)	1,585±384	1,505±283	n.s.
Creatinine (mg/dl)	0.9±0.3	0.6±0.1	0.05
Insulin (μU/ml)	6.6±5.0	7.3±4.7	n.s.
Renin (ng/ml/h)	1.4±2.0	0.6±0.3	n.s.
Aldosterone (pg/ml)	79±39	66±22	n.s.
Epinephrine (pg/ml)	49±28	30±25	0.05
Norepinephrine (pg/ml)	382±128	347±123	n.s.
Fasting blood sugar (mg/dl)	99±14	111±30	n.s.
HbA1c (%)	5.3±0.4	5.8±1.4	n.s.
Total cholesterol (mg/dl)	182±27	204±30	0.05
HDL cholesterol (mg/dl)	57±15	57±13	n.s.
Triglycerides (mg/dl)	88±60	111±68	n.s.

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; n.s., not significant.

the brachial and ankle arterial pressure wave (ΔT) was determined by the foot-to-foot method based on wave front velocity theory. The distance between the arm and ankle (D) was calculated based on anthropometric data for the Japanese population (17). Brachial-ankle PWV was calculated as $D/\Delta T$.

Finally, we studied blood pressure and autonomic nervous response to mental stress. ECG was monitored using a standard lead II. A digital photoplethysmographic device (Finapres 2300; Ohmeda, Englewood, USA) was attached to the right middle finger to obtain blood pressure measurements. Systolic and diastolic blood pressures (SBP/DBP) and the RR interval were recorded in a beat-to-beat manner for 10 min with subjects at rest, during a mental stress test that consisted of counting backwards, and after the stress test (15, 19, 20). The examiner read out a five-figure number and the subjects were requested to recite it in reverse order as quickly as possible. This procedure was repeated using different number combinations for 6 min. The blood pressure and RR interval fluctuated in a beat-beat manner. To obtain reliable data, we calculated the mean value for a 256-s period under each condition. The cardiovascular response to mental stress was examined in terms of the differences between the mean values during stress and those at rest. These data will be used for another study employing spectral analysis.

Off-line analysis was later performed on a personal computer (PC 9801-RX; NEC, Tokyo, Japan). The trendgrams of

the RR interval, SBP, and DBP were visually inspected on the computer display. A 256-s segment without movement of artifacts or premature ventricular contractions was selected for the final analysis. To study the autonomic contributions to cardiovascular regulations, we examined the frequency components of cardiovascular variability using spectral analysis. The 10-s oscillations of blood pressure, known as Mayer waves, are mediated mainly by the α -adrenergic system, whereas the respiratory-related oscillations of the heart rate are mediated purely by the vagus nerve. Those parameters were used as measures of sympathetic vascular and cardiac vagal modulations, respectively (21, 22). As such, we calculated the Mayer wave-related (0.07–0.14 Hz) and respiratory-related (0.15–0.40 Hz) absolute power spectra for each parameter according to previous reports (21, 22). Baroreflex sensitivity was examined by transfer function analysis of blood pressure and RR interval variabilities (22, 23) according to previous reports.

Statistical Analysis

All data are expressed as the mean±SD. Patients were classified into high (7.3 nmol/ml or over) and low (7.2 nmol/ml or below) homocysteine groups according to the median value. An unpaired Student's *t*-test was used for intergroup comparisons of the baseline data. Correlation coefficients were cal-

Table 4. Finger Blood Pressure and Autonomic Nervous Functions at Rest, during Stress and Recovery

	Baseline		Stress		Recovery		Group × period interaction	Group effect	Period effect
	Low group	High group	Low group	High group	Low group	High group			
SBP (mmHg)	112.8±20.9	123.8±17.4	131.8±22.5	149.5±19.2 [†]	113.1±20.9	129.2±16.1 [†]	n.s.	*	***
RR interval (ms)	867.1±136.0	924.7±138.6	804.9±118.3	830.8±118.3	845.9±134.5	901.6±136.3	n.s.	n.s.	***
SBP Mayer power (mmHg ²)	3.2±2.3	3.1±2.2	11.1±7.4	11.4±6.9	3.5±2.1	3.1±2.6	n.s.	n.s.	***
Baroreflex sensitiv- ity (ms/mmHg)	6.9±4.5	11.4±5.4	4.4±1.4	8.2±5.3 [†]	5.7±2.8	9.6±4.4 [†]	n.s.	**	***
RR respiratory power (ms ²)	153.9±267.4	404.8±451.4	105.5±87.2	357.8±416.1	157.2±313.2	289.3±305.1	n.s.	n.s.	n.s.

SBP, systolic blood pressure; n.s., not significant. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, [†] $p<0.05$ vs. Low group.

culated by Pearson's product-moment. Multiple regression analysis was used to assess independent associations between one dependent and two or more independent variables. Sex was used as a dummy variable (1=male, 2=female). Differences in frequency were tested by χ^2 analysis. Repeated measure analysis of variance (ANOVA) was performed to examine the group effect (low homocysteine group vs. high homocysteine group), the period effect (baseline, stress and recovery), and the interaction between the two. Post hoc analyses were performed using Student's *t*-test (paired or unpaired). Analysis of covariance (ANCOVA) was used to adjust age and sex. *p* values <0.05 were considered statistically significant.

Results

Table 1 shows the clinical characteristics of the studied subjects. Higher brachial-ankle PWV was associated with older age ($r=0.64$, $p<0.001$), higher concentration of plasma homocysteine ($r=0.35$, $p<0.05$) and higher SBP ($r=0.62$, $p<0.001$) (Table 2). Moreover, plasma homocysteine concentration correlated positively with plasma creatinine concentration ($r=0.66$, $p<0.001$) and negatively with total cholesterol ($r=-0.35$, $p<0.05$). Multiple regression analysis showed that both age ($\beta=0.478$, $p=0.05$) and plasma homocysteine concentration ($\beta=0.388$, $p=0.01$) were independent contributors to the brachial-ankle PWV.

In the comparison between the high and low homocysteine groups, men were more common in the high homocysteine group while women were more common in the low homocysteine group (Table 3). Age tended to be lower in the high homocysteine group than in the low homocysteine group, although the difference did not reach the level of statistical significance ($p=0.164$). Plasma creatinine concentration was higher ($p=0.05$) and total cholesterol concentration was lower ($p=0.05$) in the high homocysteine group, although other lipid profiles and glucose metabolism did not differ. Resting blood pressures, heart rate, and PWV did not differ

between the two groups. Plasma renin activity, aldosterone and norepinephrine concentrations were similar between the two groups, although epinephrine concentration was slightly higher ($p=0.05$) in the high homocysteine group.

SBP increased during stress compared with the resting condition in both the high and low homocysteine groups (Table 4, Fig. 1). SBP was higher during the stress and recovery periods in the high homocysteine group than in the low homocysteine group, although baseline blood pressure did not differ. Sympathetic vasomotor tone as assessed by the Mayer wave power of blood pressure variability increased during stress compared with the baseline values and returned to the baseline level in both groups. Baroreflex sensitivity and the respiratory power of RR interval variability at rest tended to be higher in the high homocysteine group than in the low homocysteine group, possibly due to the slightly younger age in the former. Baroreflex sensitivity was significantly reduced during stress in both the high and low homocysteine groups and tended to return to the baseline level during recovery. However, baroreflex sensitivity was significantly higher during the stress and recovery periods in the high homocysteine group.

Discussion

We compared blood pressure response to mental stress between hypertensive patients with high plasma homocysteine concentration and those with low plasma homocysteine concentration. SBP during and after mental stress was significantly higher in patients with a high homocysteine concentration than those with a low concentration, although baseline blood pressures were similar between the two groups. These differences were significant even after adjusting for sex and age. Thus high plasma homocysteine could enhance the blood pressure response to mental stress.

Blood pressure response to mental stress is regulated by several factors. Autonomic nervous mechanisms may be the most deeply involved. In our study, the baseline norepineph-

Fig. 1. Finger systolic blood pressure at rest, during stress and recovery in high homocysteine (closed bars) and low homocysteine (open bars) groups. * $p < 0.0001$, † $p < 0.05$.

rine concentration was similar between the high and low homocysteine groups. Moreover, sympathetic vasomotor response during stress and after recovery did not differ between the two groups. Thus, enhanced blood pressure reactivity to stress is not due to increased sympathetic vasomotor activity. Involvement of the impairment of the depressor neural mechanism is less likely, because baroreflex sensitivity during stress was higher in the high homocysteine group than the low homocysteine group. In other words, the depressor mechanism functioned to suppress the enhanced blood pressure response to stress in the high homocysteine group.

Another possibility is an increased Na reabsorption in the kidney due to insulin resistance (10, 11). However, plasma insulin and fasting glucose concentrations were similar between the two groups. Moreover, plasma renin activity tended to be higher in the high homocysteine group than the low homocysteine group. Thus the possibility of increased sodium retention due to insulin resistance is also less likely.

Finally, the most reasonable and probable explanation may be a direct action of homocysteine on the arterial wall. Previous data showed that hyperhomocysteinemia can induce smooth muscle proliferation (6), endothelial dysfunction (7), collagen synthesis, and deterioration of elastic material of the arterial wall (8), all of which could increase arterial stiffness. In fact, our data showed that plasma homocysteine concentration independently and positively correlated with brachial-ankle PWV, a measure for systemic arterial stiffness. Large arteries can instantaneously accommodate the volume of blood ejected from the heart, storing part of the stroke volume during systolic ejection and draining this volume during diastole. This cushioning function, or Windkessel property, prevents a rapid increase in SBP. In other words, stiffening of the arterial system increases early systolic peak in response to a similar degree of ventricular ejection. We believe that hyperhomocysteinemia could predominantly increase the stiffness of the large elastic arteries rather than that of the muscular arteries, leading to a diminution of the Windkessel property.

Our data are consistent with the previous report showing that plasma homocysteine concentration is positively associated with aortic PWV in hypertensive patients (9).

Another unique finding of this study was the prolonged high blood pressure after stress in patients with hyperhomocysteinemia. Because the heart rate after stress was similar between the two groups, the prolonged high blood pressure cannot be attributed to increased cardiac output, but rather must be due to increased systemic vascular resistance. These data suggest that hyperhomocysteinemia may be associated with functional abnormalities in the small arteries and/or arterioles. It has been shown in rats that mild hyperhomocysteinemia is associated with stiffer small arteries that have increased collagen deposition (24). Increased small artery stiffness may delay the recovery of systemic vascular resistance after stress.

Our study has a very important clinical implication. Blood pressure reactivity to stress has been reported to be related to organ damage or prognosis in patients with hypertension (14, 25). The strategy of reducing blood pressure reactivity to stress is thus an important aspect for hypertension treatment. This study raises the possibility that a strategy to reduce plasma homocysteine concentration could increase arterial elasticity and decrease blood pressure reactivity to stress in hypertensive patients. To clarify this hypothesis, we need a prospective, interventional study.

Our study has potential limitations. We studied treated patients, and thus medications may have affected the plasma homocysteine and cardiovascular responses to stress. In fact, the blood pressures were within the normal range in 16 out of 50 patients. However, the frequency of normotension did not differ between the high homocysteine and low homocysteine groups. Moreover, the types and rate of use of antihypertensive, antidiabetic and antidyslipidemic medications were similar between the two groups. Thus, it seems less likely that medications could have seriously modified the results. It is now considered ethically problematic to perform a complete washout of medications. Moreover, increased blood pressure variability is of clinical significance not only in untreated but also in treated hypertensive patients (26, 27). Thus it is of clinical significance to study treated hypertensive patients as well.

In conclusion, hyperhomocysteinemia was associated with a more stiffened arterial system and increased blood pressure reactivity to stress in treated hypertensive patients. The strategy of reducing plasma homocysteine may reduce arterial stiffness and blood pressure reactivity to stress. To clarify this hypothesis, we need a prospective, interventional study.

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