OPEN

Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males

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Hyperhomocystemia has been reported to be associated with cardiovascular disease, especially stroke. The resistive index (RI) estimated by carotid ultrasound is an established variable for estimating the risk of cerebral infarction. The aim of this study was to evaluate the relationship between homocysteine concentration and carotid RI, a marker of cerebral vascular resistance in essential hypertensive patients. We measured serum total homocysteine and carotid RI in 261 patients. Multiple linear regression analysis was used to determine the association of homocysteine with carotid RI and intima media thickness (IMT). Age, sex, BMI, systolic blood pressure (SBP), homocysteine, total cholesterol, high density lipoprotein-cholesterol (HDL-C), uric acid, CRP, HbA1c, estimated glomerular filtration rate, and use of antihypertensive agents were included as independent variables. Age, sex, use of antihypertensive agents, HDL-C and homocysteine levels were shown to be significant predictors of carotid RI, but not IMT. Multiple regression analysis in men older than 65 years showed homocysteine and SBP were associated significantly with carotid RI. In elderly male patients, homocysteine was the strongest predictor of carotid RI (B = 0.0068, CI = 0.0017–0.0120, P = 0.011) in the multivariate model. In conclusion, hyperhomocysteinemia is associated with carotid RI, a surrogate marker of cerebral vascular resistance, especially in elderly men.

H omocysteine is a naturally-occurring amino acid produced from a methyl donor, methionine and essential for intracellular metabolism[1]. Several epidemiological studies have shown that high levels of total homocysteine are associated with an increased risk of atherosclerotic disease[2–6]. However, the association between homocysteine and atherosclerosis has not been observed consistently[7–9] and the mechanisms by which hyperhomocysteinemia increases cardiovascular risk in humans remains controversial[10,11]. Furthermore most clinical trials to lower homocysteine levels using folic acid or multivitamin treatment have failed to reduce cardiovascular events[12–14].

Ultrasound of the carotid artery measures structural, functional, and hemodynamic alterations and is used widely as a marker of atherosclerosis[15,16]. Prospective population-based studies in Europe and United States have reported that carotid intima media thickness (IMT) measured by ultrasound is associated positively with subsequent occurrence of stroke[17,18]. The resistive index (RI) calculated using carotid artery Doppler ultrasound is an established variable for estimating the risk of cerebral infarction as it reflects downstream arterial resistance[19]. We reported previously that changes in carotid hemodynamics of patients with essential hypertension were associated with asymptomatic white and gray matter lesions visualized on magnetic resonance imaging[20] A recent study showed that homocysteine levels were also associated with aortic stiffness in elderly subjects, with this relationship being particularly strong in very elderly subjects[21]. Lim et al. reported an association between homocysteine and pulsatility index which has been used as an index of vascular resistance in the intracranial artery of patients with ischemic stroke[22]. To date, the relationship between serum homocysteine levels and vascular resistance in hypertensive patients remains unclear. This study evaluated the association between homocysteine concentration and cerebral vascular resistance estimated by carotid RI and IMT in patients with essential hypertension, with a special focus on elderly men.

Table 1 | Clinical characteristics of the study subjects (n = 261)

	Total	Men aged ≥ 65 yr (n = 52)	P-value
Number (male/female)	261 (126/135)	52 (52/0)	<0.001
Homocysteine (μmol/L)	9.8 ± 4.7	10.7 ± 3.5	0.230
Age (yr)	61.8 ± 12.9	72.9 ± 5.2	<0.001
BMI (kg/m²)	25.1 ± 3.6	24.6 ± 2.5	0.403
SBP (mmHg)	150.0 ± 21.2	149.5 ± 17.8	0.085
DBP (mmHg)	88.2 ± 16.2	82.3 ± 10.9	<0.001
PR (/min)	68.3 ± 11.3	63.7 ± 9.6	<0.001
Total cholesterol (mg/dL)	197.1 ± 35.4	185.1 ± 34.7	<0.001
HDL-cholesterol (mg/dL)	54.3 ± 15.2	51.1 ± 13.8	0.161
TG (mg/dL)	128.2 ± 73.1	115.1 ± 48.4	0.215
HbA1c (%)	5.6 ± 0.8	5.7 ± 0.9	0.510
eGFR (mL/min/1.73 m²)	72.8 ± 15.6	67.2 ± 18.1	0.024
Uric acid (mg/dL)	5.7 ± 1.4	6.1 ± 1.3	0.036
CRP (mg/dL)	0.08 (0.04–0.16)	0.07(0.04–0.18)	0.370
uAlb/Cr (mg/gCr)	18.0 (9.1–60.4)	16.1(9.3–39.9)	0.862
Mean IMT (mm)	0.78 ± 0.19	0.87 ± 0.18	0.001
Carotid RI	0.68 ± 0.08	0.73 ± 0.06	<0.001
Antihypertensive agents	113(43.3%)	23(44.2%)	0.099
Ca channel antagonist	89(34.1%)	26(50.0%)	0.030
ACE inhibitor	16(6.1%)	5(9.6%)	0.359
ARB	48(18.4%)	8(15.4%)	0.606
Diuretic	7(2.7%)	3(5.8%)	0.248
Beta-blocker	23(8.8%)	3(5.8%)	0.468
Alpha-blocker	4(1.5%)	0(0.0%)	0.369
Hypoglycemic agents	2(0.8%)	0(0.0%)	0.527
Lipid-lowering agents	18(5.0%)	1(0.0%)	0.170

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; TC, total cholesterol; HDL, high-density lipoprotein; TG, triglyceride; HbA1c, hemoglobin A1c; eGFR, estimated-glomerular filtration rate; CRP, C-reactive protein; uAlb/Cr, urinary albumin/creatinine; IMT, intima-media thickness; RI, resistive index; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

Methods

Study population. The study was a single-center, cross-sectional study of 261 consecutive patients with essential hypertension who attended an outpatient clinic at Ehime University Hospital. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, measured three times in the sitting position using a brachial sphygmomanometer, or taking an antihypertensive drug. Patients with a previous history of symptomatic cardiovascular disease, myocardial infarction, valvular heart disease, symptomatic cerebrovascular events, thyroid disease, glomerulonephritis, estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m², renovascular hypertension, or abnormalities in kidney structure were excluded from the study. Elderly was defined as aged ≥ 65 years and young as aged < 64 years. Increasing age and male sex are known strong determinants of serum homocysteine levels[23] with elderly patients assumed to have a higher risk for asymptomatic, clinically significant, stenosis of carotid bifurcation[24]. Accordingly, these patients are recommended to have imaging of the carotid bifurcation[24]. We therefore focused on elderly men in our study.

The study was approved by the ethical committee of Ehime University Hospital, with informed consent being obtained from each patient prior to enrolment. All experiments were performed in accordance with relevant guidelines and regulations.

Blood and urine sampling and laboratory measurements. A fasting blood sample was taken from the brachial vein after the patient had rested in the supine position for 30 min. Serum creatinine, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) uric acid, and C-reactive protein (CRP) were measured using automatic enzyme immunoassays. Glycated hemoglobin (HbA1c) was analyzed by high-performance liquid chromatography (HPLC) on an Adams - A1c HA-8160 (Arkray Inc., Kyoto, Japan). Serum total homocysteine was measured using HPLC and was carried out by SRL Inc. (Tokyo, Japan). Estimated glomerular filtration rate (eGFR) was calculated using the Japanese equation for eGFR: 194 × Cr^-1.094 × age^-0.287 (×0.739 (female))[25]. Urinary albumin and Cr concentration were determined using an automated analyzer (Architect c8000; Toshiba, Inc), and the albumin to Cr ratio (uAlb/Cr) calculated to evaluate urinary albumin excretion.

Table 2 | Multiple regression analysis of risk factors for carotid resistive index (RI) in the total study group (n = 261)

	Unstandardized coefficients			
Independent factors	B	SE	95% confidence interval	P value
Age	0.0019	0.0004	0.0012–0.0027	<0.0001
Sex (male)	0.0302	0.0108	0.0515–0.0089	0.0057
BMI	0.0022	0.0013	−0.0005–0.0048	0.1058
SBP	0.0001	0.0002	−0.0005–0.0004	0.8672
Total cholesterol	−0.0002	0.0001	−0.0005–0.0001	0.1884
HDL-cholesterol	0.0008	0.0003	0.0002–0.0015	0.0148
HbA1c	−0.0034	0.0066	−0.0163–0.0096	0.6082
eGFR	0.0006	0.0003	−0.0001–0.0012	0.0534
Uric acid	0.0049	0.0040	−0.0030–0.0128	0.2225
CRP	−0.0034	0.0231	−0.0479–0.0422	0.8840
Homocysteine	0.0020	0.0010	0.0001–0.0040	0.0390
Antihypertensive agent	0.0209	0.0090	0.0032–0.0386	0.0209

R = 0.4394, Adjusted R square 0.2405, F = 5.0674 (P < 0.0001).
SE, standard error; BMI, body mass index; SBP, systolic blood pressure; HDL, HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimate-glomerular filtration ratio; CRP, C-reactive protein.

Table 3 | Multiple regression analysis of risk factors for the carotid resistive index in men aged $\geq$ 65 years (n = 52)

| | Unstandardized coefficients | | | |
Independent factors	B	SE	95% confidence interval	P value
BMI	0.0055	0.0036	−0.0018–0.0129	0.1379
SBP	0.0002	0.0003	−0.0003–0.0007	0.4616
Total cholesterol	−0.0001	0.0001	−0.0002–0.0003	0.9265
HDL-cholesterol	0.0012	0.0006	−0.0001–0.0025	0.0608
HbA1c	0.0024	0.0102	−0.0182–0.0231	0.8139
eGFR	−0.0005	0.0005	−0.0015–0.0005	0.3279
Uric acid	−0.0090	0.0064	−0.0221–0.0040	0.1680
CRP	0.0558	0.0439	−0.0330–0.1446	0.2111
Homocysteine	0.0068	0.0026	0.0017–0.0120	0.0110
Antihypertensive agent	0.0237	0.0166	−0.0099–0.0572	0.167

R = 0.5882, Adjusted R square 0.1661, F = 1.9237 (P = 0.0651).
SE, standard error; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimate-glomerular filtration ratio; CRP, C-reactive protein.

Ultrasound evaluation. Ultrasound evaluation of the CCA was performed as described previously[26] using an alpha-7 (Aloka Co., Tokyo Japan) that incorporated a 7.5-MHz probe equipped with a Doppler system. After the subjects had rested in the supine position for at least 10 min with their neck in a slightly hyperextended position, optimal visualization of the right CCA was performed. The IMT of the far wall was measured in the CCA at two sites, 1 and 2 cm proximal to the bulb from the anterior, lateral, and posterior approaches. The mean IMT of the measurements was then calculated. Doppler evaluation was performed by scanning the right CCA in the anterior projection, with the sample volume at the center of the vessel determined using color-flow mapping. The carotid RI was calculated using the following formula: max velocity-minimum velocity/max velocity[27]. Two physicians carried out the ultrasound evaluation. The inter-observer coefficient of variation (CV) for mean IMT was 7.7% and intra-observer CV 6.2%. For the RI, inter-observer CV was 9.7% and intra-observer CV 5.5%.

Statistical analysis. All the analyses were performed using SPSS 18.0 (SPSS, Inc., IL, USA). All the data were expressed as mean $\pm$ standard deviation (SD), with the exception of CRP and uAlb/Cr which were expressed as median value and interquartile ranges (25% and 75% percentiles). Comparison of the clinical characteristics between the total study group and men aged $\geq$ 65 years was performed using unpaired t tests. The Mann-Whitney U test was used to compare CRP and uAlb/Cr in the two groups, while the $\chi 2$ test was used to analyze the distribution of drug use. The effect of independent parameters on carotid RI and mean IMT was examined by multivariate linear regression analysis, with age, sex, body mass index (BMI), SBP, serum levels of homocysteine, total cholesterol, HDL-C, uric acid, CRP, and HbA1c, eGFR, and use of antihypertensive agents included as the independent variables. M multiple regression analysis was also performed on men aged $\geq$ 65 years using the same independent variables, except for age and sex. Values of p < 0.05 were considered statistically significant.

Results
Patient characteristics. The clinical characteristics of the patients are summarized in Table 1. DBP, pulse rate, total cholesterol, and eGFR were lower in men aged $\geq$ 65 years compared with the total group, whereas uric acid level, IMT, and carotid RI were higher. The use of antihypertensive drugs, statins, and antiplatelet drugs was similar in the two groups.

Association between clinical factors and carotid RI. A multivariable regression model of the carotid RI in the total study group showed that age, sex, serum levels of HDL-C, use of antihypertensive agents, and homocysteine were associated significantly with carotid RI, whereas BMI, SBP, total cholesterol, HbA1c, eGFR, uric acid, and CRP were not (Table 2). In the analysis of men aged $\geq$ 65 years, carotid RI was shown to be associated significantly with homocysteine and SBP, but not with BMI, total cholesterol, HDL-C, HbA1c, eGFR, uric acid, CRP or use of antihypertensive agents (Table 3). In elderly male patients, homocysteine was the strongest predictor of carotid RI in the multivariate model (B = 0.0068, 95% confidence interval = 0.0017–0.0120, P = 0.011).

Association between clinical factors and mean IMT. In contrast to the above results, multivariate regression analysis of the total study group showed age, serum levels of HDL-C were independent significant variables of mean IMT, whereas sex, BMI, SBP, total cholesterol, HbA1c, eGFR, uric acid, CRP, homocysteine, and use of antihypertensive agents were not (Table 4). In elderly male patients, no variable reached statistical significance (Table 5).

Discussion
This study used carotid RI as a surrogate marker of cerebral peripheral artery resistance and showed that the index was associated

Table 4 | Multiple regression analysis of risk factors for mean intima-media thickness in the total study population (n = 261)

| | Unstandardized coefficients | | | |
Independent factors	B	SE	95% confidence interval	P value
Age	0.0051	0.0011	0.0029–0.0074	<0.0001
Sex (male)	0.0201	0.0325	−0.0842–0.0440	0.5376
BMI	−0.0027	0.0040	−0.0106–0.0052	0.5059
SBP	0.0007	0.0007	−0.0006–0.0020	0.2867
Total cholesterol	0.0003	0.0004	−0.0005–0.0011	0.4421
HDL-cholesterol	−0.0021	0.0010	−0.0040–−0.001	0.0402
HbA1c	0.0162	0.0197	−0.0227–0.0552	0.4127
eGFR	−0.00139	0.00096	−0.00327–0.00050	0.150
Uric acid	−0.0078	0.0121	−0.0317–0.0160	0.5174
CRP	−0.0267	0.0697	−0.1641–0.1107	0.7021
Homocysteine	0.0022	0.0030	−0.0036–0.0080	0.4562
Antihypertensive agent	0.0416	0.0271	−0.0117–0.0950	0.1256

R = 0.4599, Adjusted R square 0.163, F = 4.2920 (P < 0.0001).
SE, standard error; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimate-glomerular filtration ratio; CRP, C-reactive protein.

Table 5 | Multiple regression analysis of risk factors for mean intima-media thickness in men aged $\geq$ 65 years (n = 52)

	Unstandardized coefficients			
Independent factors	B	SE	95% confidence interval	P value
BMI	0.0179	0.0124	−0.0071–0.0430	0.1556
SBP	−0.0001	0.0009	−0.0019–0.0016	0.8866
Total cholesterol	0.0001	0.0004	−0.0008–0.0009	0.9074
HDL-cholesterol	0.0015	0.0021	−0.0028–0.0058	0.4891
HbA1c	0.0599	0.0344	−0.0096–0.1294	0.0890
eGFR	−0.0009	0.0017	−0.0043–0.0024	0.5704
Uric acid	0.0083	0.0219	−0.0360–0.0525	0.7082
CRP	0.0478	0.1500	−0.2552–0.3507	0.7518
Homocysteine	−0.0060	0.0084	−0.0229–0.0109	0.4772
Antihypertensive agent	0.0551	0.0567	−0.0594–0.1696	0.3364

R = 0.405, Adjusted R square −0.039, F = 0.8063 (P = 0.6237).
SE, standard error; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimate-glomerular filtration ratio; CRP, C-reactive protein.

significantly with homocysteine levels in patients with essential hypertension, especially elderly male patients.

A high RI is associated with a large difference in velocity between the systolic and diastolic phases detected by pulse-wave Doppler ultrasound[19]. This difference in flow velocities reflects downstream resistance, which could, in part, depend on the degree of stiffness of the peripheral arteries. Staub et al. reported the carotid RI was a predictor of cardiovascular mortality and morbidity, at least comparable to the well-established IMT[19]. We also demonstrated an association between carotid hemodynamics such as diastolic perfusion rate and asymptomatic white and gray matter lesions in patients with essential hypertension[20]. Taken together, these reports suggest that carotid hemodynamic indices such as RI and diastolic perfusion rate are markers of cerebrovascular stiffness. The data of the present study also showed that carotid RI is associated independently with age, sex, HDL-C, use of anti-hypertensive medication, and homocysteine levels. This finding suggests that hyperhomocysteinemia is a risk factor for cerebrovascular stiffness.

Interestingly, the factors associated significantly with mean IMT were age and HDL-C, but not serum homocysteine levels. We reported previously that carotid RI was associated with increased serum levels of IL-6 and CRP in hypertensive patients, whereas mean IMT was not. We also reported that treatment of hypertensive patients with an angiotensin II receptor blocker for 24 months improved carotid hemodynamics and diastolic perfusion rate, but not mean IMT[28]. Taken together, these findings indicate the carotid RI is a useful marker of arterial stiffness, while mean IMT is a useful marker of atherosclerosis.

In other words, carotid RI and arterial stiffness may be influenced and altered by inflammation (IL-6 and CRP), oxidative stress (homocysteine[29]) and medications. In our study, HDL-C showed an independent, negative association with mean IMT but a positive association with carotid RI. The reason for this difference could not be determined, but may have been due to the different nature of the two parameters, one being hemodynamic and the other a result of structural alterations.

An association between hyperhomocysteinemia and atherosclerotic disease was first suggested more than 40 years ago[30]. Subsequently, numerous cross-sectional and case control studies have demonstrated a clear association between total serum homocysteine and the presence of coronary, carotid, and peripheral vascular disease[2–6]. However, a systemic review of 12 randomized controlled trials involving 47,429 participants showed that homocysteine-lowering interventions compared with placebo did not significantly affect non-fatal or fatal myocardial infarction, stroke or death by any cause[31–33]. Clinical benefit of homocysteine lowering therapy is very controversial topics. One possible reason why these therapies were ineffective for preventing cardiovascular diseases is

thought to be related to confounding factors such as anti-hypertensive drugs, statins, and antiplatelet drugs[34]. In the homocysteine-lowering trials, anti-hypertensive drugs, statins and antiplatelet drugs were used by 30–70%, 60–80% and 80–90% of participants, respectively. Another possible reason why homocysteine-lowering multivitamin therapy appears to be ineffective for preventing cardiovascular diseases may be related to the age of study participants. The present study showed that serum homocysteine levels in elderly male patients showed a marked and highly significant association with carotid RI. This suggests that high serum homocysteine may be a marker of an increase in RI in these elderly patients, who are at an even greater risk of stroke. However, to date there have been no randomized clinical trials on homocysteine-lowering therapy in patients older than 65 years. Further randomized clinical trials are therefore needed to evaluate the effect of homocysteine –lowering therapy on cardiovascular disease in elderly hypertensive patients.

The present study had several limitations including a relatively small study population. In addition, because of its cross-sectional design we could not determine the mechanism for the relationship we observed between homocysteine and carotid RI, especially in elderly male patients with hypertension.

In conclusion, this study shows that serum homocysteine level in elderly male patients with essential hypertension is a strong predictor of carotid RI, a surrogate marker of cerebral peripheral artery resistance.

1. Mudd, S. H. et al. Homocysteine and its disulfide derivatives: A suggested consensus terminology. Arterioscler Thromb Vasc Biol. **20**, 1704–1706 (2002).
2. Nyfard, O. et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. **337**, 230–236 (1997).
3. Soinio, M. et al. Elevated plasma homocysteine level is an independent predictor of coronary heart disease events in patients with type 2 diabetes mellitus. Ann Intern Med. **140**, 94–100 (2004).
4. Cheng, S. W., Ting, A. C. & Wong, J. Fasting total plasma homocysteine and atherosclerotic peripheral vascular disease. Ann Vasc Surg. **11**, 217–223 (1997).
5. Perry, I. J. et al. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet. **346**, 1395–1398 (1995).
6. Towfighi, A., Saver, J. L., Engelhardt, R. & Ovbiagele, B. Factors associated with the steep increase in late-midlife stroke occurrence among US men. J Stroke Cerebrovasc Dis. **17**, 165–168 (2008).
7. Selhub, J. et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med. **332**, 286–291 (1995).
8. McQuillan, B. M., Beilby, J. P., Nidorf, M., Thompson, P. L. & Hung, J. Hyperhomocysteinemia but not the c677t mutation of methylenetetrahydrofolate reductase is an independent risk determinant of carotid wall thickening. The perth carotid ultrasound disease assessment study (cudas). Circulation. **99**, 2383–2388 (1999).
9. Kim, S. J. et al. Biomarkers of asymptomatic carotid stenosis in patients undergoing coronary artery bypass grafting. Stroke. **42**, 734–739 (2011).
10. Maron, B. A. & Loscalzo, J. The treatment of hyperhomocystenemia. Annu Rev Med. **60**, 39–54 (2009).

11. Loscalzo, J. Homocysteine-mediated thrombosis and angiostasis in vascular pathobiology. *J Clin Invest.* **119**, 3203–3205 (2009).
12. Toole, J. F. *et al.* Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* **291**, 565–575 (2004).
13. Lonn, E. *et al.* Heart outcomes Prevention Evaluation (HOPE) 2 Investigators: Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* **354**, 1567–1577 (2006).
14. Bønaa, K. H. *et al.* NORVIT Trial Investigators: Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med.* **354**, 1578–1588 (2006).
15. Cardoso, C. R. *et al.* Factors associated with carotid intima-media thickness and carotid plaques in type 2 diabetic patients. *J Hypertens.* **30**, 940–947 (2012).
16. Simon, A. *et al.* Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. *J Hypertens.* **20**, 159–169 (2002).
17. O'Leary, D. H. *et al.* Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med* **340**, 14–22 (1999).
18. Chambless, L. E. *et al.* Carotid wall thickness is predictive of incident clinical stroke. The Atherosclerosis Risk in Communities (ARIC) study. *Am J Epidemiol* **151**, 478–487 (2000).
19. Staub, D. *et al.* Prediction of cardiovascular morbidity and mortality: comparison of the internal carotid artery resistive index with the common carotid artery intima-media thickness. *Stroke.* **37**, 800–805 (2006).
20. Kurata, M., Okura, T., Watanabe, S. & Higaki, J. Association between carotid hemodynamics and asymptomatic white and gray matter lesions in patients with essential hypertension. *Hypertens Res.* **28**, 797–803 (2005).
21. van Dijk, S. C. *et al.* Homocysteine level is associated with aortic stiffness in elderly: cross-sectional results from the B-PROOF study. *J Hypertens.* **31**, 952–959 (2013).
22. Lim, M. H., Cho, Y. I. & Jeong, S. K. Homocysteine and pulsatility index of cerebral arteries. *Stroke* **40**, 3216–3220 (2009).
23. Jacques, P. F. *et al.* Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr.* **69**, 482–489 (1999).
24. Ricotta, J. J. *et al.* Up dated society for vascular surgery guidelines for management of extracranial carotid disease. *J Vasc Surg.* **54**, e1–e31 (2011).
25. Matsuo, S. *et al.* Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis.* **53**, 982–992 (2009).
26. Manabe, S., Okura, T., Watanabe, S. & Higaki, J. Association between carotid haemodynamics and inflammation in patients with essential hypertension. *J Hum Hypertens.* **19**, 787–791 (2005).
27. Rifkin, M. D. *et al.* Evaluation of renal transplant rejection by duplex Doppler examination: value of the resistive index. *Am J Roentgenol.* **148**, 759–762 (1987).
28. Okura, T. *et al.* Long-term effects of angiotensin II receptor blockade with valsartan on carotid arterial stiffness and hemodynamic alterations in patients with essential hypertension. *Clin Exp Hypertens.* **30**, 415–422 (2008).
29. Hoffman, M. Hypothesis: hyperhomocysteinemia is an indicator of oxidative stress. *Med Hypotheses.* **77**, 1088–1093 (2011).
30. McCully, K. S. & Ragsdale, B. D. Production of arteriosclerosis by homocysteinemia. *Am J Pathol.* **61**, 1–11 (1970).
31. Marti-Carvajal, A. J. *et al.* Homocysteine-lowering interventions for preventing cardiovascular events. *Cochrane Database Syst Rev.* **1**, CD006612 (2013).
32. Spence, J. D. & Stampfer, M. J. Understanding the complexity of homocysteine lowering with vitamins: The potential role of subgroup analyses. *JAMA.* **306**, 2610–2611 (2011).
33. Spence, J. D. B vitamin therapy for homocysteine: Renal function and vitamin B12 determine cardiovascular outcomes. *Clin Chem Lab Med.* **51**, 633–637 (2013).
34. Debreceni, B. & Debreceni, L. Why do homocysteine-lowering B vitamin and antioxidant E vitamin supplementations appear to be ineffective in the prevention of cardiovascular diseases? *Cardiovasc Ther.* **30**, 227–233 (2012).

Author contributions

Additional information