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Original Article

Lack of Association Between Total Serum Homocysteine and Extracranial Cerebral Flow

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Background/Purpose: High homocysteine (Hcy) concentration is associated with slow coronary flow. This study examined the association between Hcy and hemodynamic status in the extracranial cerebral arteries in healthy individuals.

Methods: A total of 535 healthy adults underwent physical examination and duplex ultrasonography of the extracranial carotid and vertebral arteries, and blood laboratory tests, including biochemistry and serum total Hcy. Flow hemodynamic parameters including velocity, resistance, and volume of the carotid and vertebral arteries were measured. Multiple regression analysis was performed to examine the association between Hcy and the flow parameters.

Results: Participants with higher Hcy were more likely to have a lower systolic velocity of the internal carotid artery ($p = 0.01$) and vertebral artery ($p < 0.001$), and lower resistance of the vertebral artery ($p = 0.004$). However, the multiple-adjusted means of the flow velocity, resistance, and flow volume of the carotid or vertebral artery were not significantly different across quartiles of Hcy. When Hcy was treated as a continuous variable, there was still no significant relationship between Hcy levels and the aforementioned hemodynamic status.

Conclusion: Our results did not support the hypothesis that the levels of Hcy are associated with the flow velocity, resistance, and volume of the extracranial cerebral artery in healthy individuals.

Key Words: blood flow velocity, carotid artery, vertebral artery Doppler duplex ultrasonography, homocysteine

Homocysteine (Hcy) is an important factor for atherosclerosis in the large cerebral arteries.[1,2] *In vitro* evidence exists for effects of Hcy on atherogenesis and thrombogenesis. Experimental studies have shown that Hcy can be harmful to vascular smooth muscles cells and promote their proliferation. Hcy also has other possible detrimental effects.[3–9] Besides, Hcy has been reported to be

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higher in people with slow coronary artery flow than in those with normal flow velocity.[10] Although the mechanisms and relationship between Hcy and vascular hemodynamic status are not clear, hemodynamic status of slow-flow- and low-shear-stress-related vascular remodeling is one of the pathogenetic mechanisms of atherosclerosis.[11–13] Hemodynamic data including blood flow velocity, flow resistance, and flow volume can be obtained by ultrasonography.[14,15] Hcy might slow the blood flow velocity, and further induce atherosclerosis by reducing the brain flow volume. However, studies that have investigated the relationship between serum Hcy and cerebral hemodynamic status have been limited in number.

To test the hypothesis that total serum Hcy is related to cerebral slow flow, we performed a cross-sectional study with detailed clinical evaluation, serum Hcy level and duplex ultrasonography examination on the extracranial carotid and vertebral arteries in healthy participants.

Subjects and Methods

Participants

The study participants were invited from those presenting to En Chu Kong Hospital, Taipei, Taiwan for a physical health check-up between 1999 and 2007. We recruited 535 participants (56% male; mean age, 55.2 ± 14.8 years), who were free from a history of stroke, transient ischemic attack, coronary heart disease, or intermittent claudication. All participants underwent detailed questionnaires, physical examination and clinical measurements, including height, body weight, body mass index, and blood pressure. Duplex ultrasonography of the carotid and vertebral arteries, and blood laboratory tests were also performed.

Ultrasound procedure and hemodynamic measurements

Ultrasonography was performed with a Hewlett–Packard 5500 system (Philips Electronics, Bothell, WA, USA) equipped with a high-resolution broadband width linear array transducer (4–10 MHz).[16]

Participants were examined in the supine position. Images were obtained bilaterally of the proximal to distal common carotid artery (CCA), including the bifurcation, internal carotid artery (ICA), and external carotid artery. Images of the vertebral artery (VA) were obtained bilaterally from the C2–C6 segments. An experienced technologist who was blinded to the clinical data made all the ultrasound measurements.

The flow parameters that we measured were the flow velocity and resistance of the CCA, ICA and VA.[17] We also calculated the total flow volume of the VA. With regard to the flow velocity, we measured the peak systolic, end-diastolic, time average flow, and mean flow velocities. The following formula was used to calculate the mean flow velocity:[18]

$$\text{Mean flow velocity} = (\text{peak systolic velocity} + 2 \times \text{end-diastolic velocity})/3.$$

As for the resistance of the vessel, indices of resistance and pulsatility were measured. Resistance index was calculated with the formula:[19]

$$\text{Resistance index} = (\text{peak systolic velocity} - \text{end-diastolic velocity})/\text{peak systolic velocity}.$$

Pulsatility index described the shape of the waveforms. The formula for calculating pulsatility index was:[20]

$$\text{Pulsatility index} = (\text{peak systolic velocity} - \text{end-diastolic velocity})/\text{mean flow velocity}.$$

Pulsatility index and resistance index are believed to be presumptive measures of downstream vascular resistance.[18] We measured the diameter to calculate the flow volume of the VA. Color-coded flow imaging was used to measure the diameter. Flow volume was the product of flow velocity and the area in which this velocity occurred:[18]

$$\text{Flow volume (cm}^3/\text{sec)} = \text{flow velocity (cm/sec)} \times \text{area (cm}^2).$$

Laboratory analysis

After a 10-hour overnight, fast, blood samples were collected to determine with standard techniques the levels of Hcy, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, blood glucose, hemoglobin, platelets, blood urea nitrogen, creatinine, and uric acid. Venous blood samples for Hcy were collected into tubes that contained EDTA, transported to the laboratory within 30 minutes after blood drawing, centrifuged at 2000 rpm for 10 minutes, and stored separately at −20°C until analysis. Serum Hcy concentrations were measured by fluorescence polarization immunoassay (Abbott IMx System Abbott Lab, N. Chicago, IL, USA),[21,22] which correlated well with high performance liquid chromatography (r value ranging from 0.980 to 0.997).[23] The inter- and intraassay coefficients of variation of Abbott IMx Analyzer were within 10%.[23]

Statistical analysis

Populations were categorized according to Hcy quartiles. Continuous variables were presented as the mean ± standard deviation or the median, while the categorical data were presented in contingency tables. All the flow parameters in the statistical analysis were the mean values from bilateral measurements, including diameter, peak systolic velocity, end-diastolic velocity, time average flow velocity, mean flow velocity, resistance index, pulsatility index, and total flow volume. Analysis of variance and the χ^2 test were used to test the differences of the vascular risk factors and flow parameters between Hcy quartiles.

Multiple linear regression models were used to examine the relationship between Hcy and the aforementioned flow parameters. We estimated the adjusted means of the flow parameters by adjustment with age and sex, and additionally, with current smoking, systolic blood pressure, body mass index, serum creatinine, serum uric acid, and high-density lipoprotein. In the above analyses, we modeled Hcy concentrations as quartiles to avoid the assumption of linearity and to reduce the effects of outliers. To test for linear trends across Hcy quartiles, we used median Hcy concentration for each category in the multivariate model. In addition, to estimate the effects of Hcy, we calculated the odds ratio (OR) and corresponding 95% confidence interval (CI) for the change of flow parameters, according to one standard deviation increase of Hcy.

All statistical tests were two-tailed and $p < 0.05$ was considered statistically significant. Analyses were performed with SAS software (version 9.1; SAS Institute).

Results

The median (interquartile range) and mean ± standard deviation of Hcy for the 535 study participants were 9.1 (7.4–11.3) and 9.9 ± 4.9 μmol/L, respectively. The median (interquartile range) of Hcy was 7.1 (6.4–9.2) μmol/L in women compared with 10.1 (8.4–12.1) μmol/L in men ($p < 0.001$). The ranges for Hcy quartiles were: first, 1.91–7.36 μmol/L; second, 7.37–9.07 μmol/L; third, 9.08–11.32 μmol/L; and fourth, 11.33–58.7 μmol/L. Baseline characteristics of the study population according to Hcy quartiles are listed in Table 1. Higher Hcy levels were associated with older age, current smoking, higher systolic blood pressure, lower body mass index, higher serum creatinine, lower total cholesterol, and higher serum uric acid. With regard to flow parameters across Hcy quartiles, participants with higher Hcy levels were more likely to have slow systolic flow velocity in the ICA ($p = 0.01$) and VA ($p < 0.001$), slow diastolic velocity in the CCA ($p = 0.02$) and ICA ($p = 0.006$), and lower resistance index in the VA ($p = 0.004$), as shown in Table 2. The diameter and flow volume of the VA were similar across Hcy quartiles.

The least-squares mean values of the flow parameters across quartiles of Hcy, after adjustment for demographics and risk factors, are shown in Table 3. There was no significant difference in the flow velocity, resistance of the CCA, ICA, and VA, as well as the VA flow volume between different levels of Hcy. When Hcy was considered as a continuous variable, the Hcy levels were not related significantly to the aforementioned hemodynamic

Table 1. Characteristics of the study population according to homocysteine quartiles

	Quartiles of homocysteine*				p
	Q1 (n = 135)	Q2 (n = 133)	Q3 (n = 134)	Q4 (n = 133)	
Age (yr)	50	53	56	61	<0.001
Sex, male (%)	56	56	56	56	0.990
Hypertension (%)	21	13	25	25	0.130
Diabetes mellitus (%)	1.3	5.2	4.8	7.3	0.300
Current smoking (%)	27	38	48	55	0.008
Systolic blood pressure (mmHg)	120	124	128	132	<0.001
Diastolic blood pressure (mmHg)	74	74	75	78	0.110
Body mass index	29	25	25	25	0.021
Laboratory biochemical data (mg/dL)					
Fasting glucose	98	98	100	99	0.900
Serum creatinine	0.87	0.94	0.99	1.04	<0.001
Serum uric acid	6.0	5.9	6.3	6.6	0.005
Lipid profile					
Triglyceride	129	154	139	155	0.130
Total cholesterol	202	207	199	193	0.020
High-density lipoprotein	53	53	50	49	0.070
Low-density lipoprotein	133	127	122	118	0.180

Homocysteine quartiles: Q1, 1.91–7.36 µmol/L; Q2, 7.37–9.07 µmol/L; Q3, 9.08–11.32 µmol/L; and Q4, 11.33–58.7 µmol/L.

status of the VA (Table 4). The results were unchanged if we calculated the VA parameters from the left and right side separately (data not shown).

Discussion

Our results from asymptomatic adults show that Hcy concentrations were not associated significantly with flow velocity and resistance of the carotid and vertebral arteries, and the total flow volume of the VA. To the best of our knowledge, this study is the first to investigate the effect of Hcy on the hemodynamic status of the extracranial cerebral vessels.

Homocysteinemia plays an important role in the risk of atherosclerosis and stenosis on the carotid and coronary arteries.[24,25] In 1041 Framingham residents who had Hcy measurement and carotid sonography, the adjusted OR for stenosis

≥ 25% was 2.0 (95% CI = 1.4–2.9) for subjects with Hcy levels in the highest compared with the lowest quartile.[1] In the Atherosclerosis Risk in Communities Study, subjects with thickened intima–medial carotid walls (≥ 90th percentile) were more likely to have elevated Hcy levels compared with those without thickened walls (< 70th percentile).[2] Several hypotheses have been proposed to explain the harmful effects of Hcy on the large arteries.[3–9] Some recent studies have reported the effects of Hcy on hemodynamic status.[10,26–30] The relationship between hemodynamic status and vascular atherosclerosis has been established.[11–13] Zarins et al reported quantitative correlation of plaque localization with flow velocity profiles and wall shear stress.[11] In previous studies of Hcy and hemodynamic status, a significant inverse correlation between Hcy and flow velocity was reported for the coronary and ophthalmic arteries.[10,29,30] High serum Hcy concentrations is associated

Table 2. Hemodynamic parameter values of the carotid and vertebral arteries according to homocysteine quartiles

	Quartiles of homocysteine*				p
	Q1	Q2	Q3	Q4	
Common carotid artery					
Systolic velocity (cm/sec)	82.52	79.84	82.93	78.63	0.250
Diastolic velocity (cm/sec)	26.48	25.62	25.02	24.55	0.020
Resistance index	0.67	0.67	0.69	0.68	0.050
Pulsatility index	1.34	1.37	1.47	1.42	0.020
Internal carotid artery					
Systolic velocity (cm/sec)	66.76	63.05	64.53	60.78	0.010
Diastolic velocity (cm/sec)	31.00	29.33	29.53	27.91	0.006
Resistance index	0.53	0.53	0.54	0.53	0.770
Pulsatility index	0.81	0.82	0.86	0.83	0.770
External carotid artery					
Systolic velocity (cm/sec)	70.09	68.35	71.81	71.92	0.260
Diastolic velocity (cm/sec)	14.12	14.76	15.03	14.68	0.350
Resistance index	0.79	0.78	0.79	0.79	0.990
Pulsatility index	1.95	1.89	1.98	1.99	0.240
Vertebral artery					
Systolic velocity (cm/sec)	44.40	40.50	38.80	35.90	<0.001
Diastolic velocity (cm/sec)	17.60	16.40	16.20	15.30	0.190
Resistance index	0.57	0.52	0.51	0.47	0.004
Pulsatility index	0.94	0.87	0.85	0.79	0.012
Flow volume (cm³/sec)	100.90	98.40	96.40	88.70	0.130

*Homocysteine quartiles: Q1, 1.91–7.36 μmol/L; Q2, 7.37–9.07 μmol/L; Q3, 9.08–11.32 μmol/L; and Q4, 11.33–58.7 μmol/L.

with the slow flow phenomenon in non-stenotic coronary artery. Barutcu et al investigated the relationship between Hcy and the coronary slow flow phenomenon.[31] Other researchers also have found elevated levels of plasma Hcy in patients who have angiographically proven normal coronary artery with slow flow in symptomatic[10,30] and asymptomatic[28] adults. Hyperhomocysteinemia impairs coronary flow velocity reserve in experimental studies.[26] The mechanisms of coronary slow flow are still not clear. Serum Hcy-related endothelial dysfunction and oxidative stress are suggested to be the causes of coronary slow flow.[10,26] Although there was increasing evidence of the Hcy effect on slow flow, our study demonstrated that there was no such association in the extracranial cerebral arteries in asymptomatic adults.

In terms of study design, the aforementioned studies on Hcy and flow hemodynamic status were small case–control studies.[10,30] The largest previous study had 50 participants in the control group and 53 in the case group.[10,26] Our study measured the flow parameters and serum Hcy levels in a total of 535 participants. The larger sample size in our study gave it greater statistical power. The absence of an association between Hcy concentrations and flow parameters was found after full adjustment for age and sex and other vascular confounding factors. These results remained consistent when Hcy was analyzed as quartile categories or as a continuous variable. One experienced technician, who was blind to the clinical data, performed all the duplex ultrasonography examination of all the participants in the study, without the bias of inter-observer reliability.

The possible reasons why our results were different from those in previous studies are as follows. First, the study target populations differ between

Table 3. Adjusted mean values of flow parameters of the carotid and vertebral arteries across homocysteine quartiles

Parameters	Quartiles of homocysteine* – Model 1[†]				p	Quartiles of homocysteine* – Model 2[‡]				p
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
Common carotid artery										
Systolic velocity (cm/sec)	81.74	79.41	82.00	81.20	0.92	79.32	77.58	80.02	79.85	0.03
Diastolic velocity (cm/sec)	25.43	25.30	25.21	25.90	0.51	25.55	25.10	25.36	26.06	0.48
Resistance index	0.68	0.68	0.69	0.68	0.70	0.67	0.67	0.68	0.67	0.74
Pulsatility index	1.39	1.38	1.43	1.39	0.93	1.33	1.36	1.39	1.34	0.96
Internal carotid artery										
Systolic velocity (cm/sec)	65.11	62.60	65.18	62.44	0.39	64.15	60.09	65.58	60.56	0.39
Diastolic velocity (cm/sec)	29.82	28.99	29.87	29.18	0.71	29.99	28.37	30.50	29.19	0.83
Resistance index	0.54	0.53	0.54	0.53	0.19	0.53	0.53	0.53	0.52	0.24
Pulsatility index	0.83	0.83	0.86	0.81	0.42	0.80	0.80	0.83	0.78	0.57
Vertebral artery										
Systolic velocity (cm/sec)	41.1	38.7	39.5	40.2	0.72	37.4	35.3	37.5	38.8	0.35
Diastolic velocity (cm/sec)	16.1	15.6	16.5	17.3	0.17	14.9	14.5	16.2	17.4	0.04
Resistance index	0.53	0.50	0.52	0.51	0.48	0.49	0.47	0.48	0.49	0.82
Pulsatility index	0.89	0.84	0.86	0.86	0.53	0.80	0.77	0.80	0.80	0.81
Flow volume (cm^3/sec)	92.8	94.1	98.2	99.4	0.12	87.7	90.9	94.6	97.0	0.13

*Homocysteine quartiles: Q1, 1.91–7.36 μmol/L; Q2, 7.37–9.07 μmol/L; Q3, 9.08–11.32 μmol/L; and Q4, 11.33–58.7 μmol/L; [†]model 1: adjusted for age and sex; [‡]model 2: model 1 plus current smoking, systolic blood pressure, serum creatinine, body mass index, uric acid, and high-density lipoprotein.

the studies. Participants in previous studies were selected from those who were suspected to have coronary artery disease, whereas our study subjects were free from symptoms of vascular diseases. Compared with our asymptomatic population, people with coronary symptoms might have different life style, risk factors, and medication, which could change the Hcy-related effects on blood flow. The effects of Hcy in different categories of population might not be the same. The present study of symptom-free subjects might not be applicable entirely to diseased individuals. Second, measurement of flow velocity in the extracranial cerebral arteries in the present study and coronary artery in other studies was different. The coronary flow was earlier measured by angiography,[10,26–28,31] whereas carotid or vertebral artery flow in our study was measured by duplex ultrasonography. The absolute values of flow velocity might differ with various methods of measurement, and that possibly accounted for the difference between the results in

the present and previous studies. Compared with ultrasonography, angiography might underestimate the presence of atherosclerotic plaques.[10] Besides, flow volume and flow resistance could not have been measured in the previous coronary artery studies. Intravascular ultrasonography, which could overcome this limitation, was not performed in these studies.[10,26–28,31] Our study involving ultrasonography provided more precise hemodynamic data than did the previous studies.

Another possible explanation for our different results is that our target vessels were the arteries to the brain, and not the heart. The hemodynamic patterns of the cerebral and cardiac vessels are different because of their different physiological mechanisms.[15] The flow patterns on duplex ultrasonography of the peripheral vessels, including the cardiac arteries, are triphasic waveforms with high resistance, whereas the carotid and vertebral arteries show low resistance flow, to provide adequate blood supply to the brain, and the brain

Table 4. Relations of plasma homocysteine to the flow parameters of the carotid and vertebral arteries

Parameters	Regression coefficients* – Model 1[†] (95% CI)	p	Regression coefficients* – Model 2[‡] (95% CI)	p
Common carotid artery				
Systolic velocity (cm/sec)	−0.12 (−1.52 to 1.28)	0.87	−0.92 (−2.90 to 1.04)	0.36
Diastolic velocity (cm/sec)	0.04 (−0.45 to 0.52)	0.89	0.02 (−0.68 to 0.71)	0.96
Resistance index	−0.0003 (−0.006 to 0.005)	0.91	−0.003 (−0.010 to 0.004)	0.40
Pulsatility index	0.006 (−0.02 to 0.03)	0.66	−0.009 (−0.05 to 0.68)	0.61
Internal carotid artery				
Systolic velocity (cm/sec)	−1.15 (−2.66 to 0.37)	0.14	−1.42 (−3.52 to 0.67)	0.18
Diastolic velocity (cm/sec)	−0.42 (−1.12 to 0.29)	0.24	−0.35 (−1.38 to 0.68)	0.50
Resistance index	−0.002 (−0.008 to 0.003)	0.46	−0.005 (−0.013 to 0.002)	0.17
Pulsatility index	−0.001 (−0.017 to 0.015)	0.90	−0.007 (−0.029 to 0.015)	0.52
Vertebral artery				
Systolic velocity (cm/sec)	0.24 (−1.00 to 1.50)	0.71	0.89 (−0.88 to 2.67)	0.32
Diastolic velocity (cm/sec)	0.35 (−0.31 to 1.02)	0.29	0.82 (−0.18 to 1.82)	0.11
Resistance index	0.0005 (−0.02 to 0.02)	0.95	0.002 (−0.02 to 0.02)	0.89
Pulsatility index	0.003 (−0.03 to 0.03)	0.83	0.0005 (−0.04 to 0.04)	0.98
Flow volume (cm³/sec)	2.3 (−1.06 to 5.69)	0.18	3.8 (−0.95 to 8.62)	0.12

*Linear regression coefficients represent difference of hemodynamic parameters of the carotid artery for one standard deviation increment of plasma homocysteine; [†]model 1: adjusted for age and sex; [‡]model 2: model 1 plus current smoking, systolic blood pressure, serum creatinine, body mass index, uric acid, and high-density lipoprotein. CI = confidence interval.

has richer collateral circulation than any other organ.[15] Besides, the blood flow in the brain is more constant than in the heart among healthy individuals. This is because the brain autoregulatory system can prevent syncope while blood pressure fluctuates during daily living.[32] This is possibly because the different collateral circulation and hemodynamic status of the brain and heart cause different effects of Hcy on the blood flow. Therefore, clinically, the effects of Hcy on cardiac and cerebral vascular disease might not be the same.[33,34]

The study limitations are as follows. First, our study lacked information on some determinants of total Hcy levels, such as dietary patterns, food folic acid fortification, and vitamin supplements. The use of a single Hcy measurement to classify persons might have underestimated the strength of any association, because of regression dilution.[35] Second, the extracranial carotid and vertebral arteries that we sampled might not indicate completely the hemodynamic status of the intracranial

cerebral blood flow. The results of our study might not be extrapolated entirely to the whole brain circulation. Using transcranial Doppler sonography could be a better way for assessing cerebral hemodynamics. However, a poor ultrasound window with failure of penetration through the skull is not uncommon in older people. Third, the flow volume of the CCA and ICA was not measured according to the protocol for carotid duplex ultrasonography for participants undergoing a physical health check-up. Thus, as for the carotid artery, we could only examine the relationship of Hcy and flow velocity. Besides, carotid or vertebral artery stenosis with plaques can lead to a change in hemodynamic status. In the present study, we could not perform subgroup analysis on the flow parameters of the sampled arteries with and without stenosis or plaques. Furthermore, the flow volume and velocity might have been reduced by VA hypoplasia. We could not clearly distinguish whether the VA was stenotic or hypoplastic.

In conclusion, we found that the flow velocity, volume and resistance of the extracranial cerebral arteries were not associated significantly with the serum concentration of Hcy in asymptomatic individuals. This differs from the effects of Hcy on coronary flow. Further prospective studies investigating the intracranial cerebral arteries in diseased individuals are needed to confirm the role of Hcy in the hemodynamic flow of the brain.

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