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Original Research

Cerebral blood flow velocity is associated with endothelial function in men

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Abstract

Background and objective: Reduction in cerebral blood flow with aging leads to cognitive decline and brain atrophy. Cerebrovascular hemodynamics are associated with vascular function. However, little is known about endothelial function in relation to cerebral blood flow at rest. The present study aimed to examine the association between microvascular endothelial function and middle cerebral blood flow.

Material and methods: This study involved 60 healthy middle-aged and elderly men. The microvascular endothelial function was measured via digital reactive hyperemia index using pulse amplitude tonometry, and the mean middle cerebral blood flow velocity and cerebrovascular conductance were measured using transcranial Doppler ultrasonography.

Results and conclusions: Reactive hyperemia index was significantly correlated with the mean middle cerebral blood flow velocity and cerebrovascular conductance. Multiple regression analysis further indicated that the correlation was significant after adjustment of covariates, such as age, body mass index, smoking status, medication history, blood pressure, and arterial stiffness. Further, Reactive hyperemia index was found to be a significant independent determinant of the mean middle cerebral blood flow velocity and cerebrovascular conductance. The present study demonstrated that vascular endothelial function is associated with cerebral blood flow and is an independent potential confounding factor in healthy middle-aged and older men.

Keywords

Vascular function; Brain; Blood flow

1. Introduction

Cerebral blood flow decreases with advancing age [1, 2]. Cerebral hypoperfusion impairs oxygen delivery and metabolism, leading to neurodegeneration, apoptosis, and cognitive dysfunction [3]. Reduced cerebral blood flow is associated with poor cognitive function [4]. Cerebral blood flow is lower in patients with dementia than in the healthy population, and reduction in cerebral blood flow is a risk factor for cerebrovascular diseases and cerebral atrophy [5]. Cerebrovascular disease pathogenesis is related to vascular deterioration, including hypertension, arterial stiffness, and endothelial dysfunction, leading to cognitive decline [6]. Aging also has a harmful effect on systemic vascular function in humans [7]. Vascular dysfunction is thought to increase the hemodynamic forces transmitted to peripheral organs,

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resulting in microvascular damage [8, 9]. The relationship between vascular health and cerebrovascular hemodynamics seems to be of clinical and research interest in identifying efficacious markers of brain aging.

Vascular functions, such as arterial stiffness and endothelial function, play critical roles in regulating blood flow and pressure, and brain function [10, 11]. Although it is well established that the progression of vascular dysfunction increases the risk of cardiovascular and cerebrovascular diseases [12, 13], the relationship between vascular endothelial function and intracranial cerebral blood flow is not clear. A few studies have demonstrated a relationship between vascular function and white matter hyperintensity or neurological activation during cognitive tasks [6, 14]. However, no studies have examined endothelial function in relation to cerebral blood flow. This study aimed to investigate the vascular endothelial function and cerebral blood flow in middle-aged and older healthy men by evaluating vascular endothelial function, and cerebral blood flow velocity and cerebrovascular conductance of middle cerebral artery.

2. Methods

2.1 Ethics

All potential risks associated with the study were explained to the subjects, and written informed consent was provided by all participants. All procedures were reviewed and approved by the Ethics Committee of the University of Tsukuba.

2.2 Subjects

We recruited middle-aged and older men over 40-year of age through local newspaper advertisements. We excluded individuals who had undergo treatment for cardiovascular and cerebrovascular diseases and those having a history of neurological diseases, or mood disorders. Sixty individuals were finally included in this study.

2.3 Study procedures

All experiments were conducted in the morning after a 12 h overnight fast. Subjects abstained from alcohol and caffeine for at least 12 h and exercise for at least 24 h before the experiments. Measurements were taken in a quiet, temperature-controlled room (at 24–26 °C) after a rest period of at least 20 min. Cerebral blood flow, endothelial function, and other cardiovascular hemodynamics and risk factors were assessed.

2.4 Cerebral blood flow

The cerebral blood flow velocities of the right middle cerebral arteries were measured using a 2 MHz transcranial Doppler ultrasonography system (Ez-Dop; DWL Elektronische Systeme, Sipplingen, Germany) [15]. Doppler signals were obtained by adjusting the position of the maximal reflected signal over the temporal window, and the probe was fixed using a headband. The subjects were instructed to breathe normally and avoid holding their breaths. The end-tidal pressure of carbon dioxide ($P_{ET}CO2$) was monitored by a nasal cannula (Nihonkoden Ltd., Tokyo, Japan). Data were collected with a sampling frequency of 1000 Hz and analyzed offline (Lab Chart 7, AD instruments, Springs, CO, USA). Mean middle cerebral blood flow velocity (MCAVmean) was obtained from a stable phase of >5 min. Cerebrovascular conductance index (CVC) was calculated as MCAVmean divided by mean arterial blood pressure.

2.5 Endothelial function

The microvascular endothelial function was evaluated by finger plethysmography using pulse amplitude tonometry (Endo-PAT 2000, Itamar Medical Ltd., Caesarea, Israel). Two-finger probes were placed on the index finger of each hand, which included a system of inflatable latex air cushions within a rigid external case. After baseline measurement for 5 min, the forearm was occluded by inflating a blood pressure cuff to >60 mmHg of the individual's systolic blood pressure on the test arm for 5 min, followed by deflating to induce reactive hyperemia for 5 min. The contralateral arm was used as the control arm. The digital pulse wave amplitude was recorded continuously during baseline, occlusion, and reactive hyperemia following the cuff deflation. The reactive hyperemia index (RHI) was calculated automatically using a system software algorithm [16]. RHI is the ratio of the average amplitude of post- and pre-occlusion in both fingers and at the baseline level.

2.6 Cardiovascular hemodynamics and risk factors

The carotid-femoral pulse wave velocity (cfPWV) was measured as a marker of arterial stiffness using a semi-automated vascular access system, as previously described [17]. Carotid and femoral pressure waveforms were obtained using two applanation tonometry sensors incorporating an array of 15 transducers (Form PWV/ABI, Colin Medical Technology, Komaki, Japan). The distance between the left common carotid and left femoral arterial recording sites was divided by the transit time to obtain the cfPWV. Simultaneously, the brachial systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate were assessed by an electrocardiogram and an oscillometric extremity cuff.

Blood samples were collected from the antecubital vein after overnight fasting. Plasma and serum were collected and stored at −80 °C, until the assay was performed. Serum total cholesterol and low density lipoprotein (LDL) cholesterol, triglyceride, and plasma glucose levels were determined using the standard enzymatic techniques provided by a commercial laboratory (LSI Medience Corporation Ltd., Tokyo, Japan).

2.7 Statistical analyses

All variables were expressed as the means ± standard deviation (SD). The Pearson correlation coefficient was used to determine the association between RHI, MCAVmean and CVC, and partial correlation. Multiple regression analysis was performed to determine the independent correlation between MCAVmean and CVC, and variables. The covari-

ates were entered as dependent variables in the subsequent multiple linear regression analysis. Statistical data analyses were performed using the SPSS software (Version 24; IBM, Armonk, NY, USA), and statistical significance was set at $P < 0.05$ a priori, for all comparisons.

3. Results

The characteristics of the participants are presented in Table 1. As shown in Fig. 1, RHI was significantly correlated with MCAVmean and CVC (r = 0.274 and r = 0.273, respectively; $P < 0.05$). A significant correlation between RHI and MCAVmean and CVC was evident after adjustments for age, body mass index, total and LDL cholesterol, triglyceride and glucose level, $P_{ET}CO2$, smoking status, and medication history (partial r = 0.322, and partial r = 0.296, respectively; $P < 0.05$). Table 2 shows the results of the multiple linear regression analyses considering age, body mass index, medication use, smoking, status, LDL cholesterol level, cardiovascular hemodynamics, and RHI. The results revealed that MCAVmean and CVC were independently determined by cfPWV (β = –0.388 and β = –0.364, respectively; $P < 0.05$) and RHI (β = 0.296 and β = 0.285, respectively; $P < 0.05$) after adjusting for the entered covariates.

TABLE 1. The characteristics of the study participants.

Variables	Means		SD
Anthropometric characteristics			
Age, years	62	±	9
Height, cm	167	±	7
Weight, kg	65	±	10
Body mass index, kg/m^2	23	±	3
Cardiovascular hemodynamics			
Heart rate, bpm	59	±	8
Systolic blood pressure, mmHg	129	±	16
Diastolic blood pressure, mmHg	81	±	10
Mean arterial blood pressure, mmHg	97	±	11
cfPWV, cm/s	1048	±	225
Reactive hyperemia index, %	2.2	±	0.6
Middle cerebral blood flow velocity			
Systolic peak flow velocity, cm/s	83	±	19
Diastolic end flow velocity, cm/s	38	±	9
Mean flow velocity, cm/s	56	±	13
End-tidal pressure CO_2, mmHg	39	±	4
Other parameters			
Total cholesterol, mg/dL	210	±	33
LDL cholesterol, mg/dL	125	±	32
Triglyceride, mg/dL	108	±	45
Glucose, mg/dL	105	±	28
Anti-hypertensive medication, n (%)	10 (17%)		
Lipid lowering medication, n (%)	6 (10%)		
Smoking, n (%)	6 (10%)		

LDL, low density lipoprotein; cfPWV, carotid-femoral pulse wave velocity; CO_2, carbon dioxide.

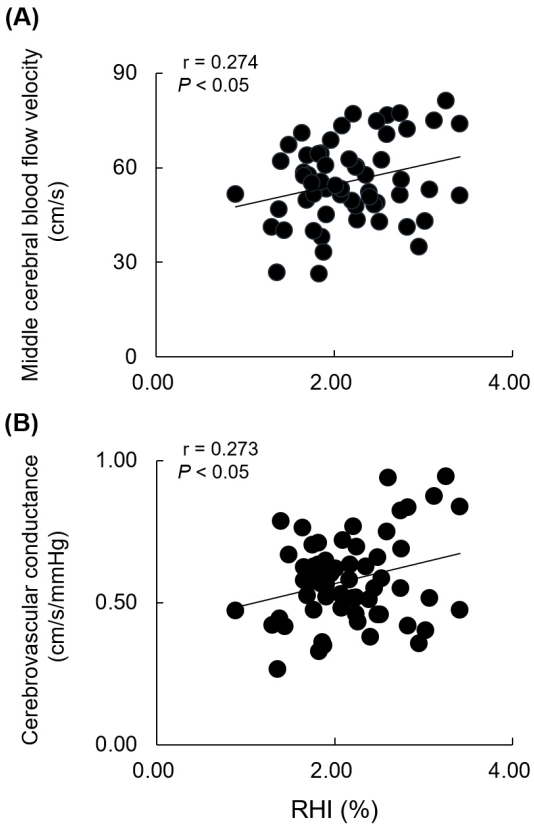

FIG. 1. The relationship between reactive hyperemia index (RHI). (A) middle cerebral blood flow velocity and (B) cerebrovascular conductance.

4. Discussion

This study investigated the relationship between endothelial function and cerebral blood flow in healthy middle-aged and older men. We observed that RHI was significantly correlated with MCAVmean and CVC. Multiple regression analysis revealed a significant independent association between RHI and MCAVmean and CVC after adjustment for covariates including age, body mass index, smoking status, medication history, and cardiovascular hemodynamics. The results of the present study suggest that monitoring vascular endothelial function in addition to arterial stiffness is important for the management of cerebral hemodynamics in middle-aged and older men.

Endothelial dysfunction is considered a key factor in the progression of cerebrovascular diseases, including lacunar and ischemic stroke [18, 19]. Impaired vasodilatory capacity is associated with an increased in atherosclerosis and small vessel lesions [20]. A previous study reported that endothelial function was negatively associated with white matter hyperintensity volume [13]. Another study documented that endothelial dysfunction induced cerebrovascular reactivity to carbon dioxide [21]. Furthermore, a large cohort study has demonstrated a correlation between brachial endothelial function and the intima-media thickness of the carotid artery that provides blood supply to the brain [22]. The present study expanded this notion of carotid artery to the middle cerebral artery using transcranial ultrasound Doppler,

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TABLE 2. Multivariable regression on cerebral blood flow velocity and cerebrovascular conductance.

Variable	*B*	*β*	*P*	95% CI	
				Lower	Upper
Middle cerebral blood flow velocity	R^2 = 0.276 (P = 0.045)				
Age (years)	0.295	0.213	0.213	−0.174	0.764
BMI (kg/m^2)	−0.149	−0.031	0.842	−1.647	1.349
Medication use (yes = 1, no = 0)	−4.792	−0.145	0.306	−14.108	4.523
Smoking (yes = 1, no = 0)	8.177	0.192	0.138	−2.709	19.062
LDL cholesterol (yes = 1, no = 0)	0.034	0.085	0.521	−0.073	0.142
Heart rate (bpm)	−0.042	−0.025	0.852	−0.488	0.405
Mean arterial blood pressure (mmHg)	0.152	0.127	0.384	−0.196	0.501
cfPWV (cm/s)	−0.022	−0.388	0.019	−0.041	−0.004
RHI (%)	7.034	0.306	0.022	1.055	13.012
Cerebrovascular conductance	R^2 = 0.444 ($P < 0.0001$)				
Age (years)	0.003	0.163	0.277	−0.002	0.008
BMI (kg/m^2)	−0.001	−0.010	0.941	−0.016	0.015
Medication use (yes = 1, no = 0)	−0.052	−0.132	0.291	−0.151	0.046
Smoking (yes = 1, no = 0)	0.091	0.178	0.119	−0.024	0.206
LDL cholesterol (yes = 1, no = 0)	0.000	0.065	0.578	−0.001	0.001
Heart rate (bpm)	0.000	−0.024	0.841	−0.005	0.004
Mean arterial blood pressure (mmHg)	−0.004	−0.285	0.029	−0.008	0.000
cfPWV (cm/s)	−0.0003	−0.364	0.013	−0.0004	−0.0001
RHI (%)	0.081	0.293	0.013	0.018	0.144

BMI, body mass index; LDL, low density lipoprotein; cfPWV, carotid femoral pulse wave velocity; RHI, reactive hyperemia index; CI, confidence interval.

and firstly found an association between the middle cerebral artery blood flow velocity and microvascular endothelial function independent of other cerebrovascular risk factors in healthy men. Endothelial function may play a role in regulating cerebral hemodynamics, which suggests that evaluating endothelial function using amplitude tonometry is indispensable for brain aging in men.

Several studies have reported that endothelial dysfunction is associated with cognitive decline [23]. Previous studies have reported that lower vascular endothelial function, measured by brachial flow-mediated dilation (FMD), was observed in patients with dementia and mild cognitive impairment than in healthy control subjects [24, 25]. In addition, FMD showed a worsening trend with respect to the clinical severity of dementia [24]. Another study by Gonzales *et al.* [14] reported that FMD contributed to the performance of working memory, and brain activation, evaluated by functional magnetic resonance imaging in healthy middle-aged men. In this regard, cognitive dysfunction depends on cerebral blood flow [26]. Indeed, Lucas *et al.* [27] demonstrated that blood flow velocity, measured by MCAVmean at rest, was significantly correlated with cognitive performance in the reaction time of the Stroop task in young and elderly adults. A prospective cohort study revealed that low blood flow velocity in the middle cerebral artery leads to future cognitive impairment and brain atrophy [5]. The present study demonstrated that endothelial function was associated with blood flow and conductance in the middle cerebral artery. Taken together, it is possible that endothelial dysfunction-induced cognitive impairment is mediated by cerebral blood flow.

The present study measured endothelial function using the distal microvasculature. However, the physiological mechanisms underlying the link between peripheral microvascular endothelial function and cerebral blood flow velocity were unclear in this study. Previous studies have also investigated the effect of endothelial function evaluated by FMD of the brachium on cerebrovascular outcomes and found an association with white matter hyperintensity, cerebrovascular autoregulation, and brain neurogenic activation [13, 14, 21]. Vascular endothelium maintains the vascular tone, exerts anti-atherogenic effect, and is a major regulator of vascular homeostasis [28]. Endothelial dysfunction occurs with aging, and is characterized by reduced bioavailability of the vasodilator nitric oxide (NO) and endothelium dependent hyperpolarization, which are impairment of endothelial-dependent vasodilation through reactive hyperemia [29, 30]. In a previous study, inhibition of NO induced a reduction in cerebral blood flow [31]. Another study demonstrated that the infusion of asymmetric dimethylarginine, an endogenous inhibitor of NO, reduced vascular function, increased arterial stiffness, and decreased cerebral blood flow in healthy subjects [32]. In addition, endothelial mediated vasodilation is caused by electrical coupling between the endothelium and vascular smooth muscle interaction that drive hyperpolarization by adenosine and potassium ion (K^+) [33]. Adenosine activates adenyl cyclase and protein kinase A, stimulating K^+channels in the endothelium and vascular smooth muscle, resulting in vasodilation. The activation of K^+ channel eliciting hyperpolarization contributes to dilation of cerebral parenchymal arterioles [34]. Therefore, NO

bioavailability- and hyperpolarization-related vasorelaxation could mediate the relationship between distal microvascular endothelial function and cerebral perfusion. Further studies are warranted to investigate the precise mechanism in endothelial function of cerebral arteries.

The present study has some limitations. It may be unreliable to relate endothelial function measured at the peripheral digital vasculature to the cerebral circulation. The amplitude tonometry via plethysmograph system used in the study is known to evaluate endothelial function of downstream vasculature [35]. In contrast to other techniques (e.g., ultrasound Doppler flow or strain gauge plethysmography), RHI provides ease of operation and analysis and requires minimal specific training and skill: therefore, they are suitable for use in relatively large clinical studies [36]. This method has been widely used by many investigators for predicting coronary endothelial dysfunction and cerebrovascular events [37, 38]. In terms of cerebral blood flow, the non-invasive intracranial blood flow velocity was measured by transcranial Doppler (TCD) ultrasonography in the middle cerebral artery that provides blood supply to large areas of the brain [39]. Indeed, MCAVmean has been proven to play an important role in cerebrovascular diseases, brain atrophy, and cognitive decline [5, 40]. We evaluated flow velocity rather than blood flow in this study. However, the diameter of the middle cerebral artery, as measured by magnetic resonance imaging, did not change during a large change in cerebral flow velocity [41]. Therefore, we believe that the methods used in the present study, including RHI and MCAVmean, provide an accurate measurement of microvascular endothelial function and cerebral blood flow. Furthermore, the current study did not measure gonadal hormones that could affect both endothelial function and cerebral hemodynamics [42, 43]. It is necessary to determine the effects of gonadal hormones on the relation between endothelial function and cerebral circulation in the future.

5. Conclusions

In conclusion, the current study demonstrated that microvascular endothelial function is associated with blood flow velocity and conductance of the middle cerebral artery in middle-aged and older men. These findings suggest that impaired endothelial function may be a mechanism that contributes to the development of cerebral hypoperfusion, and evaluation of endothelial function may be useful for further targeted interventions, such as life-style modifications, to manage cerebrovascular aging in men.

Abbreviations

cfPWV, carotid-femoral pulse wave velocity; CVC, cerebrovascular conductance; FMD, flow-mediated dilation; MCAVmean, mean middle cerebral blood flow velocity; NO, nitric oxide; $P_{ET}CO2$, end-tidal pressure of carbon dioxide; RHI, reactive hyperemia index.

Author contributions

NA and SM conceived and designed the experiments; NA and HK performed the experiments; NA, TY, KM and KT collected and analyzed the data. NA wrote the paper.

Ethics approval and consent to participate

All potential risks associated with the study were explained to the subjects, and written informed consent was provided by all participants. All procedures were reviewed and approved by the Ethics Committee of the University of Tsukuba (#27-68) and conformed to the principles outlined in the Declaration of Helsinki.

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Conflict of interest

The authors declare no conflict of interest.

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