Cerebral Vasoregulation in Elderly with Stroke-Protocol

The overall hypotheses for this project are:

1) Older adults with ischemic stroke have impaired cerebral vasoregulation, rendering cerebral blood flow dependent on blood pressure.

2) Autonomic blood pressure control is impaired after stroke. Activities of daily living may induce hypotension, posing a risk of hypoperfusion.

3) The distribution of impaired vasoreactivity extends beyond the infarct region into surrounding gray and white matter, affecting other vascular territories.

These hypotheses are based on the following observations. **First,** cerebral vasoregulation, which maintains steady cerebral blood flow in response to changes in systemic BP or metabolic demands, is compromised by cerebrovascular disease and further damaged by stroke.(1) In acute stroke, hypertension increases blood flow in the infarcted hemisphere, while hypotension decreases it.(2) It is not known whether cerebral vasoregulation recovers in older people with ischemic stroke, and if cerebral perfusion is different between stroke-normotensive and stroke-hypertensive subjects. **Second,** circadian blood pressure control is altered after stroke, and labile BP is associated with a worse prognosis in terms of death and disability.(3) Activities of daily living such as standing-up, eating or performing Valsalva maneuver may induce hypotension. OH is associated with impaired vasoregulation(4), and increases the risk for stroke.(5) Hypotension may lead to hypoperfusion if BP falls below an autoregulated range. **Third,** silent brain infarctions are seen on MRI in 28% of older adults with no history of stroke and in 68% of stroke victims.(6) Diffuse white matter changes and silent lacunar infarctions indicate areas of hypoperfusion.(7) Therefore impairment of vasoregulation after stroke may engage large areas of gray and white matter, affecting different vascular territories. **Our objective was to investigate cerebral vasoregulation using transcranial Doppler (TCD) ultrasound and arterial spin labeling MRI at 3 Tesla, to determine the impact of stroke on cerebral blood flow regulation and autonomic BP control in elderly people.**

Subjects

Selection criteria: This cross sectional study aimed to compare 60 subjects in the stroke-group to 60 subjects in the non-stroke group. Subjects will be 60-80 years of age, with even distribution of men and women and the racial distribution representative of greater Boston area. We enrolled (signed consent form) 172 subjects, 96 female, 91 were eligible after signing ICF and 48 controls and 43 stroke participants completed the protocol.

Stroke group: Subjects aged 60-80 years with the first large vessel hemispheric infarct affecting <1/3 of MCA territory documented by CT or MRI during acute event, defined by the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria will be included. The stroke group will include subjects with the first ever hemispheric ischemic stroke with documented neurological deficit persisting >24 hours, confirmed by radiological findings on CT and MRI. The subjects will be at least 6 months after stroke, in a clinically stable condition defined by a neurological exam, MRS<4, NIHSS <5 upon admission to the study. The eligible subjects will be alert, oriented, respond correctly to two tasks. They may have a mild deficit on the NIHSS sensory, motor and language tasks and be able to walk without assistance (MRS<4). The subjects will have to be alert, oriented and respond correctly to two tasks by the NIHSS. They will have either none or a mild deficit on the sensory, motor and language tasks. The

subjects within stroke and non-stroke groups will be stratified as “hypertensive” (BP≥140/90 mm Hg without BP controlling medications and “normotensive “(BP<140/100 without BP controlling medications).

Non-stroke group: We recruited subjects with no clinical history of stroke and no focal deficit on neurological exam. Hypertensive patients will have a diagnosis of essential hypertension and have documented BP≥140/90 mm Hg for at least 6 months. Normotensive healthy subjects with BP<140/90 mm Hg, taking no medications for BP served as controls.

Exclusion criteria: intracranial or subarachnoid hemorrhage on MRI or CT; any unstable or acute medical condition, vertebrobasilar and carotid disease diagnosed by magnetic resonance angiography (MRA) during initial work-up; dementia (by history);or inability to follow details of the protocol; diabetes; hemodynamically significant valvular disease; clinically significant arrhythmias; atrial fibrillation (if present during the study protocol), severe hypertension (systolic BP>200 and/or diastolic BP>110; or subjects taking 3 or more antihypertensive medications); current recreational drug or alcohol abuse; morbid obesity (body mass index >35); inability to obtain permission for participation from the primary care physician. In the non-stroke group, we will exclude subjects with carotid stenosis (>50% by history, Duplex ultrasound, or MRA). In the stroke group, we will exclude subjects with carotid stenosis >50% that is contralateral to the stroke or not associated with the stroke.

MRI exclusion criteria: any metallic bioimplants (including pacemakers and valve replacements), claustrophobia or inability to cooperate. TCD exclusion criteria: inability to obtain TCD signal due to poor insonation window.

Recruitment: Participants were recruited from community advertisement.

Screening visit: All subjects who are interested in this study and meet inclusion/exclusion criteria came to BIDMC CRC (Clinical research Center) and were asked to sign the informed consent and completed symptom checklist and medical history questionnaires. ECG and vital signs, height and weight were measured by a trained GCRC nurse. Heart rate, blood pressure (BP) was measured during 5 min sitting and after 1 and 3 minutes of standing. TCD insonation window was tested. Blood was drawn, to obtain a lipid profile, hematocrit and CBC. Antihypertensive medications were tapered during the 5 days prior to the study using home BP monitoring. The protocol time line and procedures are detailed below (**Table 1**).

Table 1: Protocol Time Line

Screening visit	**Day 1-3**	**Day 4-6**	**Day 7**	**Day 8**	**Day 9**	**Day 10-12**
TCD window, ECG vitals signs, blood draw Sit-to-stand test	BP Meds On Home BP monitoring	BP Meds ½ dose Home BP monitoring	GCRC admission BP Meds Off	24-hour BP monitoring Aim 2	Aim 1 - TCD-CO_2 reactivity, tilt Aim 2 - TCD-Valsalva maneuver Sit-to-stand Aim 3 - MRI study Resume BP medications Discharge from GCRC	BP Meds On Home BP monitoring

Experimental Protocol:

Subjects enrolled in the stroke and non-stroke groups were admitted to General Clinical Research Center (GCRC) for 2 day overnight stay. The structured regime that simulates everyday activities such as active standing, walking, eating and sleeping **(Table 2)** was implemented on Day 1 and Day 2**.** The subject received a standard 800 kcal meal, three times a day. Subjects rested in a comfortable sitting position during daytime. They were asked to lie down at night at 10 p.m. and wake up at 7:00 am.

See File list and table of variables (files and channels tabs for directories and filenames)

Table 2: Structured regime simulating activities of daily living for Day 1 and Day 2 admission to GCRC.

Day 1 Protocol									
Hour	7:30	9:00	11:00	12:00	13:00-16:30	17:00	18:00	19:00	22:00
Test	Breakfast	24-hour BP 24-hour ECG,EMG (Me6000)	Walk 12 min	Lunch	Sit-to-stand test Cognitive tests Beat-to-beat	Walk 12 min	Dinner	Sit-to-stand test	Sleep

		Sit-to-stand (Labview)			BP monitoring (Portapress)				
Day 2 Protocol									
	Breakfast	Head-up tilt Sit-to-stand eyes open and closed with balance COP measures	MRI	Lunch	Discharge, resume antihypertensive medications				

Day 1:

Sit-to-stand test: Heart rate, blood pressure (BP) and BFV in the MCA (blood flow velocity in the middle cerebral artery measured by Doppler ultrasound) was measured during 5 min sitting and after 1 and 3 minutes of standing. Blood was drawn, to obtain a lipid profile, hematocrit and CBC using Labview at 500 Hz. Only the first sit-to-stand test was recorded on Labview (xxxxa.dat files), the sit-to-stand tests performed in the afternoon were only recorded on 24 hour BP monitoring and ECG 24 hour monitoring (Me6000).

24-hour beat-to-beat blood pressure monitoring (ABPM): 24-hour beat-to-beat heart rate and BP monitoring sing Dynapulse was set up at 8 a.m. ABPM sleep time was set 10 pm and wake up time at 7am. BP was measured at 20 min intervals during daytime and at 30 min intervals at night.

24 hour ECG and EMG monitoring
24-hour ECG and EMG monitoring using ME6000 device (MegaElectronics) was set up at 9 a.m. to measure (ECG, EMG for 24 hours during sleep and daily activities during the protocol sit-to-stand test, walking) ME6000 directory and files) were sampled at 1000 Hz.

12 min-hallway walking: The subject walked in a circular hallway for 12 minutes to simulate normal daily walking at their usual walking speed. Level of exertion, walking distance and speed was measured.

Me6000- EMG, ECG and walk characteristics using foot switches was measured using ME6000 device and signals were capture at 1000Hz.

Pedar Mobile: Foot pressure distribution and step characteristics were measured using Pedar Mobile device that allows to measure foot pressure, force, gait timing using insoles with 99 pressure sensitive and calibrated insoles (8),(9) that allow to measure precisely foot pressure mapping during walking (Pedar directory).

Beat-to-beat BP monitoring: Beat-to-beat BP was measured from two fingers on the nondominant/non-stroke side using the Portapress–2 device (Finapres Monitoring Systems, Netherlands). This device enables continuous noninvasive BP monitoring based on photoplethysmographic volume clamp method. BP cuffs are placed on 2 fingers and measurements are done on 1 finger for 30 minutes and then switched automatically to another finger to prevent prolonged finger compression that may cause inaccuracy of the BP readings. Portapress–2 is also

equipped with a height correction device that adjusts BP readings to the arm position. Beat-to-beat BP readings will be verified every hour and before and after each test witdescription
h arterial tonometry. File directory (portapress, beatscope-program, portapres- new)

Cognitive and executive function neuropsychological testing:
All participants will be asked to complete a battery of measures assessing executive function, attention, learning and memory, mood, and activities of daily living. Stroke is associated with cognitive decline and depression that may affect activities of daily living (see source Protocol document for details).

Day 2

Head-up tilt, vasoreactivity and balance measurements

Instrumentation: Heart rate was measured using a 3-lead electrocardiogram. Beat-to-beat arterial pressure was measured from a cuff placed on the finger on the unaffected side in the stroke group or on the nondominant arm in the non-stroke group using a Portapress-2 device (FMS, Inc). Beat-to-beat BP measurements were corroborated by standard measurements of arterial pressure on the upper arm (Dynamap). Respiration will be measured using a nasal thermistor. We will measure tidal volume, end-tidal CO_2 and O_2 values, using an infrared end-tidal volume gas monitor (Capnomac Ultima, Ohmeda Inc.) attached to a face mask.
A transcranial Doppler ultrasonography system (MultiDop X4, Neuroscan, Inc.) will be used to monitor BFV in both MCAs. The right and left MCAs will be insonated from the temporal windows, by placing the 2-MHz probe in the temporal area above the zygomatic arch. Each probe will be positioned to record the maximal BFV and fixed at the desired angle using a three-dimensional positioning system attached to the light-metal probe holder. Special attention is always given to stabilize the probes, since their steady position is crucial for continuous BFV recordings. Fourier transform of the Doppler shift, a difference between the frequency of the emitted signal and its echo (frequency of reflected signal), will be used to calculate BFV. Systolic, diastolic, and mean BFV will be detected from the envelope of the arterial flow waveforms. Cerebrovascular resistance was calculated from the arterial pressure divided by BFV in the MCAs. Balance was measures suing center of pressure displacement using the force platform (Kistler, Inc) in xy z direction . Cardiovascular,respiratory and TCD signals during the tilt were recorded on Labview at 500Hz (labview, TCD directory ,xxx b.dat files) and cardiovascular,respiratory, TCD signals and balance measures were recorded at 1000 Hz (labview sit-to-stand directory). All analog signals will be recorded at 500 Hz using Labview NIDAQ (National Instruments Data Acquisition System 64 Channel/100 Ks/s, Labview 6i, Austin, TX).

Valsalva maneuver: l After rest for 5 minutes in the supine position, the subject took a breath and expire forcefully through a mouthpiece that has a small air-leak, maintaining a pressure at 40 mm Hg on a pressure gauge connected to the mouthpiece for 15 seconds. From four phases of the Valsalva maneuver, phase II and phase IV are important for the evaluation of autoregulation. In the phase II a rapid fall of blood pressure, venous return and stroke volume occur, and cerebral blood flow depends on autoregulation. In the phase IV, peripheral resistance increases leading to a blood pressure increase 30 mm Hg above baseline. All data will be continuously acquired over the period of 5 minutes during which blood pressure returns to the baseline. During phase II and phase IV, the effects of blood pressure changes on cerebral blood flow are dampened by cerebral autoregulation. The maneuver will be repeated twice.

Hyperventilation and CO_2 re-breathing: We usde hyperventilation and CO_2 re-breathing to evaluate the cerebral vasomotor range and reactivity to CO_2 change between 25-45 mm Hg. The subject rested supine for 10 minutes with continuous monitoring of heart rate, beat-to-beat BP, BFV in both MCAs, respiration, O_2 and CO_2. The subject then hyperventilated to reduce CO_2 to 25 mm Hg for 3 minutes. The subject was breathing a mixture of 5% CO_2 and 95% air from a re-breather bag to increase CO_2 above baseline to 45 mm Hg for 3 minutes, followed by a 5 minute rest to equilibrate CO_2.

Head-up tilt: After the hyperventilation/rebreathing protocol, the subject will rest supine for 10 minutes with continuous ECG, BP, TCD and CO_2, monitoring. Then the table will be tilted to 80^0 for 10 minutes. The tilt test was interrupted if symptoms of pre-syncope or blurred vision occurred, at which point the subject was returned to the supine position.

Sit-to-stand test: Active standing is a standard method for the diagnosing orthostatic hypotension (OH). Published databases show a good concordance between BP responses to tilt and standing. In elderly people, the normal response to standing-up is a transient systolic BP decline with recovery within the first minute(10), which is compensated primarily by increased peripheral resistance. OH will be diagnosed using standard criteria accepted by The Consensus Committee of the American Autonomic Society and American Academy of Neurology, as a systolic BP decline of ≥20 mm Hg or diastolic BP decline of ≥10 mm Hg within 3 minutes of standing.(11) Subjects will sit on the chair for 5 minutes with their legs elevated at 90 degrees in front of them on a stool to reduce venous pooling. The moment when both feet touch the ground will be recorded to mark the onset of standing. Subjects will stand for 5 minutes with continuous signal acquisition. Arm BP using a standard blood pressure cuff (Dynamap) will be acquired at minute 1, 3 and 5, in addition to a beat-to-beat BP at the finger positioned at the level of the right atrium. The subjects will be asked to breathe according to a metronome at their normal breathing rate.

MRI

Arterial Spin Labeling at 3T MRI: Subjects in the stroke and non-stroke group will come to the Magnetic Resonance Imaging Center at the BIDMC after completion of the TCD study. MR imaging wase performed at 3 Tesla in GE Vhi scanner with quadrature head coil. Upon arrival at the MRI center, subjects will be asked to fill out an MRI safety questionnaire and will be screened for metal objects. The subject lie down on the imaging table and the head will be stabilized within the head coil using foam padding to restrict motion artifacts. A mask placed on the subject's face will be connected to the CO_2 rebreathing circuit and the CO_2 monitor.

T1 and T2-weighted imaging: All subjects will have routine T1-weighted (spin echo) and T2-weighted fast spin echo (FSE) and Fluid-attenuation inversion recovery (FLAIR).

Continuous Arterial Spin Labeling (CASL): CASL will be done as previously described.(12),(13) A T1-weighted localization scan will be obtained before and after CASL. A scout image of the head is obtained in order to choose the appropriate location for spin labeling and flow imaging (3 minutes). CASL images will be obtained every 8 seconds and averaged over baseline, hyperventilation and CO_2 rebreathing. Flow images (with spin labeling) and control images (without spin labeling) will be then collected during baseline, hyperventilation and CO_2 rebreathing (10 minutes). Finally, a regional T1-weighted map is obtained using a modification of the spin labeling sequence (5 minutes).

Hyperventilation and CO_2 rebreathing: We will use hyperventilation and CO_2 re-breathing to evaluate cerebral vasomotor range and reactivity to CO_2 change between 25-45 mm Hg. The subject will hyperventilate to reduce

CO_2 to 25 mm Hg for 3 minutes. Then the subject will breathe a mixture of 5% CO_2 and 95% air from the rebreathing bag to increase CO_2 above baseline to 45 mm Hg for 3 minutes. The subjects will equilibrate CO_2 during supine rest.

Reference List

(1) Derdeyn CP, Grubb RL, Powers WJ. Cerebral hemodynamic impairment. Methods of measurement and association with stroke risk. *Neurology* 1999;53:251-259.

(2) Schwarz S, Georgiades A, Aschoff A, Schwab S. Effects of induced hypertension on inctracranial pressure and flow velocities of the middle cerebral arteries in patients with large hemispheric stroke. *Stroke* 2002;33:998-1004.

(3) Dawson SL, Bradley TD, Manktelow BN, Robinson TG, Panerai RB, Potter JF. Which parameters of beat-to-beat blood pressure and variability best predict early outcome after acute ischemic stroke? *Stroke* 2000;31:463-468.

(4) Novak V, Novak P, Spies JM, Low PA. Autoregulation of cerebral blood flow in orthostatic hypotension. *Stroke* 1998;29:104-111.

(5) Eigenbrodt ML, Rose KM, Couper DJ, Arnett DK, Smith R, Jones D. Orthostatic hypotension as a risk factor for stroke. The Atherosclerosis Risk in Communities (ARIC) Study, 1987-1996. *Stroke* 2000;31:2307-2313.

(6) Price TR, manolio TA, Kronmal RA, et al. Silent brain infarction on Magnetic Resonance Imaging and neurological abnormalities in community-dwelling older adults. *Stroke* 1997;28:1158-1164.

(7) Inzitari D. Leukoaraiosis: an independent risk factor for stroke? *Stroke* 2003;34:2067-2071.

(8) Hessert MJ, Vyas M, Leach J, Hu K, Lipsitz L, Novak V. Foot pressure distribution during walking in young and old. *BMC Geriatrics* 2005;5:8.

(9) Chen IH, Novak V, Manor B. Infarct hemisphere and noninfarcted brain volumes affect locomotor performance following stroke. *Neurology* 2014 Mar 11;82:828-834.

(10) Low PA, Opfer-Gehrking TL, Proper CJ, Zimmerman I. The effect of aging on cardiac autonomic and postganglionic sudomotor function. *Muscle & Nerve* 1990;13:152-157.

(11) Anonymous. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy., 46 ed 1997:1470.

(12) Detre JA, Samuels OB, Alsop DC, Gonzalez-At J.B., Kasner SE, Raps EC. Noninvasive magnetic resonance imaging evaluation of cerebral blood flow with acetazolamide challenge in patients with cerebrovascular stenosis. *J Magn Reson Imaging* 1999;10:870-875.

(13) Detre JA, Alsop DC, Vives LR, Maccotta L, Teener JW, Raps EC. Noninvasive MRI evaluation of cerebral blood flow in cerebrovascular disease. *Neurology* 1998;50:633-641.