CEREBRAL PERFUSION AND COPGNITIVE DECLINE IN TYPE 2 DIABETES

The purpose of the protocol below describes the study when it was still enrolling subjects. It is now in data-analysis only, where the data collected from the following measures is being investigated.

Our goal was to determine the mechanisms by which type 2 diabetes mellitus (DM) affects cerebral perfusion in older adults and by which uncontrolled DM contributes to cognitive impairment.

Hypothesis 1: Type 2 diabetes mellitus is associated with cerebral microvascular disease, presenting as impairments in vasoregulation and blood flow distribution, white matter abnormalities on MRI, and declined cognitive performance.
Specific Aim 1: To determine correlates of microcirculatory disturbance in type 2 DM using the measures of cerebral vasoregulation with transcranial Doppler ultrasound, regional blood flow distribution using continuous arterial spin labeling (CASL) at 3 Tesla MRI, volume and distribution of white matter changes on T2-weighted MRI and clinical cognitive and balance tasks in older adults with type 2 DM, compared to the healthy controls.
1a) To determine the effects of DM on cerebral vasoregulation, to determine the cerebral vasomotor range and CO_2 reactivity, to evaluate the dynamics of cerebral autoregulation in response to rapid BP changes.
1b) To determine the effects of DM on regional blood flow distribution, to determine the effect of DM on the volume and distribution of white matter changes (WMC), to determine the effects of WMC on blood flow distribution, we compared WMC distribution to blood flow maps.
1c) To compare cognitive and executive functions in the DM and non-DM groups we used a battery of specific psychometric measures.

Hypothesis 2: Poor glycemic control increases the severity of microvascular disease in elderly people with type 2 DM. Higher hemoglobin A1c levels are associated with reduced cerebral blood flow and more WMC and executive dysfunction. Disturbance of blood flow regulation in the fronto-temporal cortex and periventricular white matter changes contribute to executive dysfunction in type 2 DM.
Specific Aim 2: To determine association between hemoglobin A1c (HbA1c) levels and regional cerebral blood flow, volume and distribution of white matter changes, and executive function in elderly people with DM.
2a) To evaluate the effects of glycemic control on severity of microvascular disease.
2b) To characterize the mechanisms by which diabetes may lead to executive dysfunction in elderly people, and to quantify complex interactions among multiple cardiovascular, behavioral, and biochemical factors affecting cerebral blood flow.

BACKGROUND AND SIGNIFICANCE

There are 18.2 million people in the United States of all ages affected with diabetes mellitus (DM), nearly 6.3% of the population. More than 8.6 million older people (16.4%) over age 60 years had diabetes in 2004. The direct cost of diabetes epidemic was $92 billion, and an additional cost of $40 billion was for disability, work loss, and premature mortality. Diabetes increases the risk for stroke 2-4 times, doubles the mortality rate, and increases the risk for Alzheimer's disease and vascular dementia.[1]
Cardiovascular risk indices, e.g., the Framingham index, do not incorporate measures of brain function. Predictors of cerebrovascular complications of diabetes that are evidence-based on cerebral blood flow, MRI imaging, and cognitive testing are lacking. DM alters the permeability of the blood-brain barrier, thus affecting regional metabolism and microcirculatory regulation.[2] Specifically, the fronto-temporal cortex[3] and periventricular white matter[2] are most likely damaged by DM. Neuroanatomical changes in these structures affect regional perfusion, cognitive and executive functions in the elderly.[4,5] **Our goal was to determine the mechanisms by which type 2 DM affects cerebral perfusion in older adults**

and by which uncontrolled DM contributes to cognitive impairment as assessed by a clinical assessment of each patient's competence. DM may impair cerebral vasoregulation and contribute to frontal lobe dysfunction through the effects of chronic hyperglycemia on the capillary structure,[6] consequent white matter changes on magnetic resonance imaging (MRI), and regional blood flow redistribution.[7] DM management tasks are complex and difficult for elderly people. Executive dysfunction may lead to poor DM control, worsening cognitive decline and cerebrovascular morbidity. Results of this study can be implemented into broad health initiatives for prevention of cerebrovascular events and cognitive decline in elderly people with diabetes.

STUDY DESIGN AND METHODS

This study is now in data-analysis only. The protocol below reflects the procedures in place when the study was still enrolling subjects.

Study Design: Single center, cross-sectional study.

Subjects' characteristics. A total of 140 subjects were recruited from the local community with an even distribution of men and women and races representative of the greater Boston area.

Inclusion criteria:

Diabetes group: 70 men and women aged 50-85 years diagnosed with type 2 DM and treated for > 1 year.

Control group: 70 healthy subjects who were not being treated for any systemic cardiovascular, renal, or neurological disease and with no focal deficit on neurological exam, had normal glucose and HbA1c, and were normotensive (BP<140/90mm Hg) or diagnosed with hypertension.

<u>**Inclusion criteria**</u>: Diabetes group: 70 men and women aged 50-85 years diagnosed with type 2 DM and treated for > 1 year. Control group: 70 healthy subjects who were not being treated for any systemic cardiovascular (other than hypertension), renal, or neurological disease and with no focal deficit on neurological exam, had normal glucose and HbA1c, and were normotensive (BP <149/90 mm Hg) or hypertensive (BP >140/90 mm Hg).

<u>**Exclusion criteria**</u>: type I DM; any unstable or acute medical condition, myocardial infarction or major surgery within 6 months, history of a major stroke, dementia (by history) or inability to follow details of the protocol, carotid stenosis > 50% by medical history, Doppler ultrasound or by magnetic resonance angiography; hemodynamically significant valvular disease; liver or renal failure or transplant; clinically significant arrhythmias; atrial fibrillation (if present during the study protocol), severe hypertension (systolic BP > 200 and/or diastolic BP > 110 mm Hg; or subjects taking 3 or more antihypertensive medications); epilepsy; malignant tumors, current recreational drug or alcohol abuse; morbid obesity (BMI > 40); inability to obtain permission for participation from the primary care physician. MRI exclusion criteria: any metallic bioimplants (including pacemakers and valve replacements), claustrophobia, or inability to cooperate. TCD exclusion criterion was an inability to obtain TCD signal due to poor insonation window.

Protocol Overview

The experimental protocol described below was used to acquire all demographic and physiological data for Specific Aims 1 and 2. This protocol was feasible in elderly people, and admission to the General Clinical research Center (GCRC) allowed for continuous medical supervision and was outlined in the Table 1.

Table 1: Protocol Time Line

SCREENING	HOME BP	CRC ADMISSION	
	MONITORING	Day 1	Day 2
Pre-testing ECG TCD window Blood draw Physician permission	BP monitoring 4× per day	Vital signs Neurological exam Retinal imaging Cognitive testing Walking test	Transcranial Doppler Study CO_2 reactivity Valsalva maneuver, tilt, sit-to-stand MRI study Discharge from GCRC

Medications	BP meds On DM meds On	BP meds On DM meds On	BP meds hold morning before TCD and MRI DM meds On BP meds On upon completion Vitals signs before discharge

Subjects' screening and pre-tests. All subjects who meet inclusion/exclusion criteria were asked to sign the informed consent and fill out medical history and autonomic symptoms and activity questionnaires. Pre-tests: ECG, vital signs (sitting and standing heart rate and BP), height, weight, waist circumference, TCD window. Control participants were asked to perform a short psychological task (Mini-Mental State Exam described below) to assess memory and executive function. Laboratory blood tests (glucose and renal panels, HbA1c, lipid profile, hematocrit, CBC, WBC, C-reactive protein (CRP) and endothelium and pro-inflammation markers (intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule (sVCAM-1), endothelin 1(ET-1) and interleukin 1-6 (IL1-6) and angiogenic growth factors (erythropoietin (EPO), vascular endothelial growth factor (VEGF)). Permission from primary physician was contacted for pertinent medical information and their approval for participation.

Home BP monitoring. To establish BP baseline, hypertensive subjects were asked to monitor their BP at home, while on antihypertensive medications 4x per day and to call daily the investigators. The patients were asked to call or email the study coordinator with their blood pressure readings from the previous day. **Day 1 – Admission to GCRC** Subjects enrolled in the DM and control groups were admitted to BIDMC GCRC for an overnight stay. The research assistant reviewed the protocol with the subjects. The physical and neurological exams were performed. Vitals signs and glucose were measured by a GCRC nurse 4x per day.

Ophthalmologic examination was done at a separate visits[8,9]. Overnight heart rate monitoring was used to assess sleep quality and autonomic function.

Cognitive Testing:

Measures of executive function: Verbal fluency, Trail Making Test, and Clock Drawing

Verbal fluency was assessed with phonemic and semantic fluency tasks. The phonemic fluency task required the participant to generate as many words as possible beginning with a given letter (e.g., "S") for 1 minute. The semantic fluency task required the participant to generate items of a given semantic category (e.g., animals) for 1 minute. Appropriate reliability and validity had been shown in both older people and individuals with frontal lesions and norms were available. Dependent variables for the fluency measures included number of items generated for each of the three phonemic trials (i.e., F, A, S) and the number of items generated for the semantic task (i.e., animals).

The Trail Making Test (parts A and B) was a measure of shifting attention. Participants were required to sequentially connect a series of numbered circles (A), and then to alternate between numbers and letters sequentially (e.g., A-1-B-2-C-3…). Trail Making B had been shown to be sensitive to changes in executive function and frontal lobe pathology. Any participant who had not completed part B within the standard 5 minutes (300 seconds) allotted for the task was considered unable to complete the task.

Clock-in-a-Box: The Clock-in-a-Box (CIB) was a modification of the commonly used Clock Drawing Test. The CIB was designed to be a very brief and easily administered cognitive screening measure. It was also designed to be domain specific, and initial studies investigating the validity of the CIB in the identification of early cognitive problems in individuals at risk for vascular dementia indicated that it was a sensitive measure of both memory and executive function. Pilot data collected on 95 participants (48 type 2 diabetics and 47 healthy controls) suggested that the 2 CIB subscores (i.e., memory and executive subscore) were predictive of performance on the standardized measures of memory and executive function. Performance

on the CIB was based on the Executive Subscore and Memory Subscore, with a total of 4 possible points for each subscore.

Measures of attention

Digit Span (Forward and Backward subtests) was a brief task that assessed immediate memory/attention. It was administered using the standard format as described in the WAIS-III. It consists of a series of digits of increasing length, some of which were to be recited as presented (Forward subtest) and some of which were to be recited in reverse order (Backward subtest). Dependent variables (DVs) of interest for both the Forward and Backward tasks were based on the number of correctly recited digits.

Measures of learning and memory

The Hopkins Verbal Learning Test – Revised (HVLT-R) offered a *brief* assessment of verbal learning and memory (recognition and recall) that was easy to administer and score and was well-tolerated even by significantly impaired individuals. Its use had been validated with brain-disordered populations (e.g., Alzheimer's disease, Huntington's disease, amnesic disorders). The HVLT-R consisted of 12 nouns (targets) representing items from 1 of 3 semantic categories. The HVLT-R tasks included 3 learning trials, a delayed recall trial (20-25 minute delay), and a forced-choice delayed recognition trial. DVs of interest for the HVLT-R included Total Recall (total number of list items learned across trials), Delayed Recall (total number of list items recalled after the delay), Retention (percentage of items from Total Recall that were subsequently recalled on Delayed Recall), and Recognition Discrimination Index (number of list items correctly identified among non-list items). The Rey-Osterreith Complex Figure (ROCF) was a test of visual-spatial ability and visual memory. The procedure for the ROCF involved having the participant first copy the figure and then without warning immediately draw the figure from memory. Approximately 30 minutes later, participants were asked to again draw as much as they could recall about the figure. The DVs of interest were the amount of information immediately recalled (controlled for their copy score) and percentage retention. Figures such as the ROCF had been recommended to assess visual memory because they reduced the possibility that verbal mediation was used to assist recall. Normative data had been provided.

The Mini-Mental State Examination (MMSE) was a short assessment instrument used to grade cognitive mental status (orientation to time and place, registration, memory, attention and concentration, praxis, constructional and language capacity, ability to follow commands). It provided a quick and reliable quantitative assessment of cognitive state. It was designed for use with hospitalized patients and was used widely in primary care and in community-based research settings.[173] The inter-rater reliability of the MMSE in the original presentation was reported as 0.83. Performance on the MMSE was based on the total score, with a total of 30 possible points. The DV of interest for the MMSE was the total score.

Participants also completed self-report measures of mood and daily functioning. The effects of executive dysfunction on daily living were assessed using the Behavioral Assessment of the Dysexecutive Syndrome (BADS). The BADS was a 20-item questionnaire that sampled areas important to daily living likely to be affected by executive dysfunction. The BADS had been shown to discriminate healthy adults from those experiencing executive dysfunction. In addition to the BADS, participants also completed the Neuropsychological Impairment Scale (NIS) The NIS was a brief, self-report test that helped screen adults for neuropsychological symptoms. The NIS assessment could help elicit diagnostically relevant information that patients often fail to mention in a clinical interview. We also administered the Instrumental Activities of Daily Living Scale (IADL) as a traditional activity of daily living measure. This test provided valuable information about relations between cognition and various aspects of activities of daily living. Each IADL question was associated with 3 multiple choice responses, with a different value associated with each answer. Responses reflecting increased difficulty impairment were assigned a lower value (i.e., 1). The DV of interest for the IADLs was the total score, with a total of 27 possible points. Participants also completed the Geriatric Depression Scale (GDS), a self-report measure of mood. The GDS contained 30 questions

associated with overall mood, activity, sadness, and worry. Responses reflecting depressed mood were assigned a value of 1 with a total of 30 possible points. DV of interest for the GDS was the total score.

The Center for Epidemiologic Studies - Depression Scale (CES-D) was a brief, widely used, self-report, 20-item screen for symptoms of depression that had moderate correlations with the Hamilton (R = 0.44) and Raskin (R = 0.54) rating scales, and discriminated well between psychiatric in-patient and general population samples. There is high internal consistency among items, reliability coefficients range from 0.84-0.90 in different populations, and test-retest reliability ranges from 0.51-0.59 over 2-8 weeks in a representative community-dwelling population.

Retinal imaging. The Joslin Vision Network (JVN) video-digital retinal imaging system was used to document level of diabetic retinopathy and, secondarily, to identify other significant ocular and systemic abnormalities manifesting in the retina. The JVN diabetes eye care model provided a suitable retinal examination to determine level of diabetic retinopathy and macular edema.[8,9] The JVN had already been validated against the accepted standard for retinal imaging (seven-standard field 35-mm slide stereoscopic photography) and had been integrated into clinical programs in a variety of settings. The JVN included a method to diagnose equivalent clinical level of diabetic retinopathy (ETDRS) accurately and conveniently using proprietary computer software applications and a commercially available non-mydriatic retinal fundus camera with operating characteristics modified to optimize performance for low light level imaging of the retina. True color, stereoscopic, high-resolution images were obtained by certified JVN image acquisition specialists at low light levels without the need for pupil dilation. These JVN digital-video retinal images were transmitted to a central telemedicine reading center for interpretation and retinopathy severity assessment (0 = normal to 4 = severe retinopathy with additional findings).[10][11]

12 minute walk test. Subjects walked in the hallway for 12 minutes at normal walking speed with continuous measurements of gait parameters using ME6000 and Pedar Mobile that measures foot distribution using 99 sensors in the foot insole [12,13]. Foot pressure distribution (FPD). Measurement of foot pressure distribution was clinically useful for evaluation of gait pathologies and foot abnormalities in DM patients. Data was analyzed as we had previously described. Time-series pressure measurements for all 99 sensors on the foot insole were grouped into 9 anatomical masks corresponding to medial calcaneus, lateral calcaneus, medial arch, lateral arch, first metatarsal, metatarsals 2 and 3, metatarsals 4 and 5, hallux, and toes. Maximum pressure, maximum force, mean pressure, mean force, and relative load were calculated for each step and then averaged over the whole walk. Maximum pressure was defined as the greatest pressure any single sensor in each mask measured in a single step; mean pressure was defined as the average of all activated sensors in a mask for a single step. To calculate maximum and mean forces, the pressure time-series data was converted to force by multiplying each pressure value with the cross-sectional area of the corresponding sensor. The maximum force was defined as the greatest force exerted for each mask in a single step. The mean force was defined as the average force exerted in each mask for a single step. All variables were normalized by body weight and the area of each mask.

Overnight heart rate monitoring was done using a holter monitor that was set up before the 12 minute hallway walk. Subjects were asked to lie down in their bed at 10pm.

Day 2- Day 2- antihypertensive and anticholinergic medications were held on the morning before transcranial Doppler and MRI study and were resumed after the study. During their stay at the GCRC, subjects were supervised by trained GCRC nurses who monitored their BP and by the admitting physician. If BP was sustained above 180/100 mm Hg on two separate measurements, the admitting physicians made a decision whether the subject should discontinue the study or restart antihypertensive medications before the Transcranial Doppler and MRI tests and could be allowed to continue. Blood was drawn for fasting plasma glucose. Subjects checked into the SAFE laboratory at the BIDMC GCRC at 8:30 a.m., after having a light breakfast at 7:30 a.m. for transcranial Doppler study. Instrumentation for the study took 30 minutes,

and the study took 1½ hours. Physiological data during baseline supine rest, hyperventilation and CO_2 rebreathing, Valsalva maneuver, head-up tilt, and sit-to-stand tests was recorded as described below. Subjects were allowed to rest for about an hour and then MRI study was done. Subjects were discharged from GCRC upon completion of the study, if clinically stable.

Baseline: The subjects rested supine for 10 minutes with continuous monitoring of all parameters.

Hyperventilation and CO_2 rebreathing. Following instrumentation, we used hyperventilation and CO_2 rebreathing to evaluate the cerebral vasomotor range and reactivity to CO_2 change between 25 and 45 mm Hg. The subject rested supine for 10 minutes with continuous monitoring of heart rate, beat-to-beat BP, BFV in ACA and MCA, respiration, O_2, and CO_2. The subject then hyperventilated to reduce CO_2 to 25 mm Hg for 3 minutes. The subject breathed a mixture of 5% CO_2 and 95% air from a rebreathing bag to increase CO_2 above baseline to 45 mm Hg for 3 minutes, followed by a 5-minute rest to equilibrate CO_2.

Valsalva maneuver. The subjects rested for 5 minutes in the supine position. Then the subjects took a breath and expired forcefully through a mouthpiece that had a small air-leak, maintaining a pressure at 40 mm Hg on for 15 seconds. All data was continuously acquired over the period of 5 minutes during which blood pressure returned to the baseline. The maneuver was repeated twice.

Head-up tilt. The subjects rested supine for 10 minutes with continuous ECG, BP, TCD, and CO_2, monitoring. Then the table was tilted to $80°$ for 10 minutes. The subjects breathed according to a metronome set at their resting breathing frequency to minimize CO_2 changes. The tilt test was interrupted if symptoms of pre-syncope or blurred vision occurred, at which point the affected subject was returned to the supine position.

Sit-to-stand test. Balance testing was done using a sit-to-stand test. Balance was measured by center of pressure (COP) displacement using Kistler force plate in responses to sit-to-stand test with eyes open, eyes closed, and while performing a cognitive challenge as described above under the specific Aim 1a. Subjects sat on the chair for 5 minutes with continuous electrocardiogram (ECG), blood pressure (BP), transcranial Doppler ultrasound (TCD), and CO_2, monitoring with their legs elevated at 90 degrees in front of them on a stool to reduce venous pooling. Force displacement from the moment the feet touch the ground in x, y, and z directions was measured by the Kistler Force plate (Kistler Instrument Corp., Amherst, NY). Subjects stood for 3 minutes with continuous signal acquisition with eyes open. The trial was repeated with the eyes closed, and then while the subject engaged in a cognitive challenge, serial subtractions of the number 7 from 500. Arm BP using a standard blood pressure cuff (Dynamap) was acquired at minutes 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 were asked to breathe according to a metronome at a 0.15-Hz breathing rate.

All analog signals were recorded at 500 Hz using Labview NIDAQ (National Instruments Data Acquisition System 64 Channel/100 Ks/s, Labview 6i, Austin, TX) on a Pentium Xeon 2 GHz dual processor computer that was currently in use at the SAFE laboratory, and stored for offline processing. Beat-to-beat heart rate was determined from the R-wave on the ECG, and systolic and diastolic BP were determined from the corresponding maximum and minimum of the BP waveform. Systolic, diastolic, and mean blood flow velocity (BFV) were detected from the envelope of the middle cerebral artery (MCA) flow waveforms. All data was visually inspected for accuracy of R-wave detection, artifacts, and occasional extrasystoles using software written in our laboratory. Ectopy and artifacts were removed using a linear interpolation algorithm. The mean, standard deviations for R-R intervals, BP, and BFV were calculated from beat-to-beat data. Respiration and CO_2 signals were equidistantly resampled at 50 Hz.

Transcranial Doppler study took 1½ hours. Physiological data during baseline, hyperventilation and CO_2 rebreathing, Valsalva maneuver, head-up tilt, and sit-to-stand tests were recorded. A transcranial Doppler ultrasonography system (MultiDop X4, Neuroscan, Inc.) was used to monitor blood flow velocity (BFV) in anterior cerebral artery (ACA) and middle cerebral artery (MCA). The ACA and MCA were insonated from the temporal windows by placing the 2-MHz probe in the temporal area above the zygomatic arch. Each probe was positioned to record the maximal BFV and fixed at the desired angle using a 3-dimensional positioning system attached to the light-metal probe holder. Special attention was always given to stabilize the probes, since their steady position was 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), was used to calculate BFV. Systolic, diastolic, and mean BFV were detected from the envelope of the arterial flow waveforms. Cerebrovascular resistance was calculated from the arterial pressure divided by BFV in the MCAs.

MRI study was done one hour later at the Magnetic Resonance Imaging Center, in the same building as the SAFE laboratory at the BIDMC. MRI was done at 3 Tesla in a GE Vhi scanner with phase array head coil. CO_2 monitor. Vital signs, CO_2 and glucose were measured.

T1- and T2-weighted imaging. High resolution anatomical images were acquired on all subjects with the following parameters: 3D T1-weighted inversion recovery fast gradient echo (IR-FGE): $T_I = 600$ ms, $T_E/T_R = 3.3/8.1$ ms, flip angle of 10 degrees, 3 mm slice thickness, 24 cm $\times$ 19 cm Field of View (FOV), 256 $\times$ 192 matrix size; Fluid-attenuation inversion recovery (FLAIR): $T_I = 2250$ ms, $T_E/T_R = 161/11000$ ms, 5 mm slice thickness (0 mm slice spacing), 24 cm $\times$ 24 cm FOV, 256 $\times$ 160 matrix size; Dual T2-weighted fast spin echo (FSE): $T_E = 25/117$ ms, $T_R = 4000$ ms, 3 mm slice thickness, 24 cm $\times$ 18 cm FOV, 256 $\times$ 256 matrix size; 3D-MR Angiography (time of flight, TOF): $T_E/T_R = 3.9/38$ ms, flip angle of 25 degrees, 2 mm slice thickness, 20 cm $\times$ 18 cm FOV, 384 $\times$ 224 matrix . Diffusion Tensor Imaging was employed using TrackVis software (www.**trackvis.org**) to reconstruct white matter tracts. Anisotropy threshold of 0.15 and angle threshold of < 40 degree were used for reconstruction. The specific white matter tracts to be evaluated were 1) projection: cortico-spinal, cortico-bulbar and thalamic, 2) commissural: genu and splenium of corpus callosum, and 3) association: superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and uncinate fasciculus. Fractional anisotropy (FA), mean diffusivity (MD), apparent diffusion coefficient (ADC), fiber thickness and fiber density were recorded. <u>WMC—volume and distribution</u>. All FLAIR and FSE images were reviewed and scored using a clinical visual rating scale from 0 to 3.[13] The WMC volume was calculated using the automated segmentation algorithm in ACA, MCA and PCA territories. <u>Global CBF maps </u>were quantified in the gray and white matter. The two-sample t-test or the Wilcoxon test were used to compare CBF between DM and control groups for the gray and white matter. Mixed linear regression models: Dependent variables: CBF in the gray and white matter, independent variable-group, co-variants: age, sex, BMI, race, DM duration and control, BP; interactions: group and gray and white matter. To determine the <u>regional distribution of impaired vasoreactivity</u>, ACA, MCA, and PCA vascular territories were marked on CBF maps. Cerebral vasoreactivity was calculated as percentage of flow augmentation per CO_2 change between hypocapnia and hypercapnia for each vascular territory for the gray and white matter [13,14,15,16],

Subjects were discharged from GCRC upon completion of the study.

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