

STUDY DESCRIPTION

PURPOSE OF PROTOCOL

Our long-term goal was to assess whether diabetes affects the cerebrovascular response to orthostatic hypotension (OH) in older adults. Specifically, this proposal evaluated the effects of diabetic autonomic neuropathy with OH on cerebral vasoregulation using transcranial Doppler ultrasound and magnetic resonance imaging (MRI) at 3 Tesla.

Hypothesis 1: Diabetic autonomic neuropathy with OH is associated with impaired cerebral vasoregulation and cerebral blood flow depends on blood pressure.

Specific Aim 1:

1a. To determine if diabetic autonomic neuropathy with OH affects cerebral vasoregulation, we measured cerebral vasomotor range in diabetic patients with and without OH and the healthy controls.

1b. To determine the cerebral vasomotor range and endothelial CO₂ reactivity, we measured change in blood flow velocities during vasoconstriction (hyperventilation) and vasodilatation (CO₂ rebreathing) using transcranial Doppler ultrasound in the supine position.

1c. to determine if cerebral vasodilatation was effective during orthostatic stress, we measured vasomotor reserve during head-up tilt from increment of cerebral blood flow velocities with CO₂ rebreathing.

Hypothesis 2: Diabetic autonomic neuropathy with OH may predispose patients to cerebral hypoperfusion if blood pressure declines below an autoregulated range.

Specific Aim 2:

2a. To determine the effects of diabetic autonomic neuropathy on cerebral blood flow regulation, we measured the autoregulated range during rapid blood pressure fall (\approx 30 mm Hg) induced by sit-to-stand protocol and Valsalva maneuver.

Hypothesis 3: The likelihood of white matter changes on 3 Tesla MRI is increased in the presence of impaired vasoregulation and diabetic autonomic neuropathy with OH.

Specific Aim 3:

3a. To determine relationship between diabetic autonomic neuropathy and white matter changes (WMC), we used T2 weighted imaging (Fluid-attenuation inversion recovery (FLAIR) at 3 Tesla MRI. WMC volume and distribution were measured using segmentation method and compared between the three groups.

3b. To determine the relationship between WMC and impaired vasoreactivity, we used regression modeling to correlate WMC volume with vasomotor range, measured by transcranial Doppler sonography under the Specific Aim 1.

3c. To determine relationship between WMC and orthostatic hypotension, we used regression modeling to correlate WMC distribution and volume with orthostatic blood pressure. In all models, we adjusted for age, gender and duration of diabetes.

SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Diabetes is a risk factor for stroke and cardiovascular death. Diabetes is known to be associated with dysregulation of peripheral vascular system. It is not known, if cerebral vasoregulation is also impaired. This study will evaluate cerebral vasoregulation in diabetes. Many patients with diabetes develop autonomic neuropathy with orthostatic hypotension (OH) (1-3). It is not known, whether these patients also have abnormal regulation of cerebral blood flow. Clinical symptoms of OH (dizziness and cognitive decline) suggest cerebral hypoperfusion. OH has been associated with white matter changes (WMC) on MRI (4) and with strokes (5). Mounting evidence suggest that WMC reflect long-term cerebral hypoperfusion due to an underlying

cerebrovascular disease (4). **The central hypothesis for this project is that cerebral vasoregulation is impaired in diabetic autonomic neuropathy with orthostatic hypotension and that cerebral blood flow depends on blood pressure.** This proposal addresses an important issue of cerebral vasoregulation in older people with diabetes who are at the highest risk for stroke and death.

DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

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, observational study. Studies were conducted in the SAFE (Syncope and Falls in the Elderly) Laboratory at the Harvard-Thorndike Clinical Research Center, Gryzmish 818b, and at The Center for Advanced Magnetic Resonance Imaging, Dept. of Radiology at Beth Israel Deaconess Medical Center (BIDMC).

Subjects' characteristics: One hundred twenty men and women >55 and <75 years of age, with the racial distribution representative of the greater Boston area would be studied, 80 patients with diabetes and 40 controls. Diabetic patients were recruited from Joslin Diabetes Clinic and from the Harvard Cooperative Program on Aging (HCPA) research subject registry.

Inclusion criteria:

Diabetes with OH: 40 men and women aged 50-75 years with type 1 and type 2 diabetes for > 1 year who met the criteria for diabetic autonomic neuropathy with OH (BP drop >20/10 mm Hg) with autonomic symptoms score 5-10^{1-3,30,31}. Patients with diabetic autonomic neuropathy were matched with 40 diabetic patients without OH by DM duration and age + 5 years.

Diabetes without OH: 40 diabetic subjects without OH (average BP change <10/5 mm Hg over 3 minutes), autonomic symptom score 0-1 were matched with DM-OH+ group.

Controls: 40 age-matched \pm 5 years healthy subjects with no history of DM or OH served as controls and were matched with diabetes groups by age \pm 5 years.

Protocol Overview:

The experimental protocol described below was used to acquire all demographic and physiological data for Specific Aims 1, 2 and 3. 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 Visit 1	Visit 2	Visit 3
ICF PCP permission TCD window Autonomic tests Blood draw Retinal imaging	Transcranial Doppler Study CO2 reactivity Valsalva maneuver, tilt, sit-to-stand	MRI study
Medications	BP meds hold morning before TCD BP meds On upon completion	BP meds On DM meds On

	Vitals signs before discharge Pressor agents On - night prior TCD DM meds On	Vitals signs before discharge
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Visit 1 – Screening

Informed consent was obtained and the patient will fill out medical history autonomic symptoms profile and MRI safety questionnaire. The subject was asked to contact their physicians to obtain permission for participation in the study.

TCD: The insonation window was detected by placing the 2 MHz probe in the temporal area to measure blood flow velocities in the cerebral arteries. Unsuccessful insonation may occurred in up to 15% of subjects.

Autonomic tests: Deep breathing at 0.1 Hz, Valsalva maneuver and 5 minutes of tilt-up at 80o, 12 minute walk test.

Laboratory tests: Blood glucose, lipid profile, hematocrit, CBC, endothelium factors, were obtained and urine albumin-creatinine ratio were used as a marker of nephropathy.

Ophthalmologic examination: It was used for staging of retinopathy if the examination was done within the last year and the patient is clinically stable. Otherwise, ophthalmologic examination was done using the Joslin Vision Network (JVN) video-digital retinal imaging system. The goal of this component of the study was to document, at baseline, level of diabetic retinopathy and secondarily, to identify other significant ocular and systemic abnormalities manifesting in the retina. The hypothesis was that the JVN diabetes eye care model provides suitable retinal examination to determine level of diabetic retinopathy and macular edema. The JVN had validated against the accepted standard for retinal imaging, (i.e., ETDRS seven-standard field 35-mm slide stereoscopic photography) and had integrated into clinical programs in a variety of settings, including the Beetham Eye Institute at the Joslin Diabetes Center. Briefly, the JVN included a method to diagnose ETDRS equivalent clinical level of diabetic retinopathy accurately and conveniently using proprietary computer software applications and a commercially available nonmydriatic 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 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. JVN images were reviewed by certified graders who successfully complete a three-day program for provisional JVN image reader certification, followed by a six-month period of satisfactory performance under the supervision of a senior retinal specialist, who provided consultation and quality assurance overview.

The JVN component of the study was conducted at the Beetham Eye Institute at the Joslin Diabetes Center. Eligible patients wishing to participate in the study received a description of the JVN imaging process and an informed consent form to read and review. The certified JVN image acquisition specialist took a brief history and obtained digitized, nonmydriatic photographs of the patient's retina according to JVN protocol prior to pupil dilation. The retinal images obtained from the different image examination protocols were assessed to determine ETDRS level of diabetic retinopathy and recommended retinal examination follow-up based on this level of diabetic retinopathy. These evaluations were performed in the JVN Reading Center (in the BEI) environment by certified JVN readers using reading center clinical template. A copy of the JVN report was sent to the study coordinator.

Visit 2 - Evaluation of cerebral vasomotor reserve

Instrumentation: Middle (MCA) cerebral arteries and brachial artery (BRA) were insonated using 2,4 and 8 MHz probes stabilized with probe holder (Multi-Dop X4 Neuroscan, Inc.). Finapres device (Ohmeda Monitoring Systems, Englewood CO) based on photoplethysmographic volume clamp method was used for beat-to-beat BP monitoring from the finger. With adjustment for position and finger temperature, this method provided reliable estimates of arterial BP. Respiratory rate and CO₂ were measured using a mask connected to an infrared end-tidal volume monitor (Ohmeda, Capnomac Ultima). All analog signals were acquired at 500 Hz using Labview NIDAQ data acquisition system with 64 channels A/D board (National instruments, Inc.).

Protocol 1:

The subject rested supine for 10 minutes.

Valsalva maneuver: The subject in supine position performed forced expiration at a pressure of 40 mmHg for 15 seconds. This maneuver induced 20 mm Hg drop in mean BP during phase II and increased during phase IV.

Vasomotor range: The subject breathe from the bag filled with 5% CO₂ for 5 minutes. The subject will breathe at 1 cycle/sec or until CO₂ declined < 25 mm Hg for 3 minutes. The subject rested for 5 minutes on the tilt table.

Head-up tilt with CO₂ Rebreathing: The subject was tilted upright to an angle of 80° for 5 minutes, followed by a 5-minute supine rest. CO₂ rebreathing began for 1 minute and then the subject was tilted upright for 5 minutes and CO₂ rebreathing continued in upright position. CO₂ rebreathing may improve orthostatic blood tolerance, cerebral blood flow and blood pressure. The test would be interrupted if symptoms of pre-syncope occur and the patient would be returned to supine position.

Sit-to-stand: The patient were sitting for 5 minutes with legs elevated on the chair in front of them and asked to stand-up from a sitting position and stood for 3 minutes with eyes open on the force plate. This procedure was repeated with eyes closed.

Visit 3 - Distribution of white matter changes on 3 Tesla MRI

Upon arrival at the MRI center, subjects were screened using MRI safety questionnaire. The subjects were screened for metal objects on their clothes and in their pockets and asked to remove such items. The subject was given earplugs to dampen the sound made by the gradients during the imaging process. The subject was then placed inside the magnet until the part of the head was in the center. Then the study started. The MR operator was in constant two-way voice communication with the subject. The subject gave periodic updates throughout the examination and encouraged to report any discomforts immediately. Subjects were not asked to remain in the magnet more than 90 minutes and each imaging sequence took between 5-15 minutes. When the study is complete, the subject was brought out of the magnet, the coil was removed and then the subject was allowed to rest and get up slowly. Subjects were encouraged to get up slowly not to induce lightheadedness. The MRI flow images were obtained as follows:

MR imaging was done at 3 Tesla in a GE VH/I scanner. Following a quick localizing scan, T2 weighted (FLAIR-fluid attenuation inversion recovery, FSE-fast spin echo) (Flair (TR=6 sec, slices 3 mm, 256x256 (512x512), TI 2.5, echo train length 16) and T1 weighted GE (gradient

echo) images were acquired. Noninvasive magnetic resonance angiography sequence was acquired (5 min) .A scout image of the head was obtained in order to choose the appropriate location for spin labeling and flow imaging (3 min). Flow images (with spin labeling) and control images (without spin labeling) were then collected over 20 minutes. The subject breathed from the bag filled with 5% CO₂ for 5 minutes. Then the subject was instructed to breathe faster until CO₂ declines < 25 mm Hg for 3 minutes. Baseline recordings were taken for 5 minutes. Finally, a regional T1 map was obtained using a modification of the spin labeling sequence (4 min).

All images were saved to a CD-RW attached to the MRI scanner for post processing.

STATISTICAL CONSIDERATIONS

Signal conditioning: Beat-to-beat values were detected, ectopic beats and outlying values were replaced using a linear interpolation, signals were equidistantly re-sampled and detrended using polynomial filtering.

Statistical Analysis for Specific Aim 1:

The goal was to determine if cerebral vasomotor range was reduced with diabetic autonomic neuropathy and to evaluate if cerebral vasodilatation was impaired with OH.

End Points:

1. The cerebral blood flow velocities in diabetic patients with OH.
2. The cerebral vasomotor range
3. The cerebral vasodilatation in tilt.

Cerebral blood flow velocity (BFV) was measured during supine baseline, hyperventilation and CO₂ rebreathing in supine position, tilt-up and tilt with CO₂ rebreathing and compared between groups for each test using linear regression models.

Cerebral vasomotor range was calculated as percent change between the maximum at the end of CO₂ rebreathing and minimum during the hyperventilation in supine position. CO₂ reactivity was a measure of endothelial response to carbon dioxide. A regression model was fitted with BFV as the dependent variable and end-tidal CO₂ as the independent variable and BP as co-variable (11-13). The slope of resulting regression line was referred to as CO₂ reactivity, which was compared between the groups using linear regression models.

Cerebral vasodilatation with OH We measured change of BFV between supine and tilt position, and between tilt without and with CO₂ rebreathing (Figure 1A, B). With impaired vasodilatation, BFV declined with OH. CO₂ rebreathing may improve vasodilatation and increase BFV. Percent change of mean BP between supine and tilt were used an independent variable. Percent change of mean BFV will be dependent variable. Covariates that were included in this model are DM, age, gender and race, presence of OH.

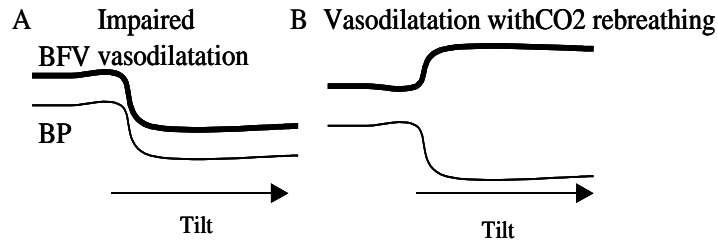


Figure 1 A shows impaired vasodilatation and BFV decline during OH.

Figure 1 B shows expected BFV improvement with CO₂ rebreathing .

Analysis: Descriptive statistics was used to summarize BFV, BP, vasomotor range and CO₂ reactivity for each group. BFV were compared between groups one-way of ANOVA. Due to possible effects of other variables, linear regression models also were used to analyze the data with BFV as the dependent variable and group as the independent variable. Covariates to be included in the model were DM duration and type, age, gender and race, OH + vs. OH-, BP. The effect of OH also were evaluated as a change of mean BP with tilt-up and during each test that was included as co-variables. Other co-variables included in the model were presence of retinopathy and nephropathy (normal vs. abnormal creatinine-albumin ratio).

Power for Specific Aim #1

Based on our preliminary data (9), the minimum difference in diastolic BFV that was of clinical interest was 9.0 cm/s to detect difference between control vs. DM-OH group during head-up tilt. Using a two-sided two-sample t-test with significance level of 0.05, it was estimated that a total sample size of 120 subjects (40 in each group) was needed to achieve 90% power to detect a difference of 9.0 cm/s in BFV during tilt with standard deviation around 11.

Statistical Analysis for the Specific Aim 2:

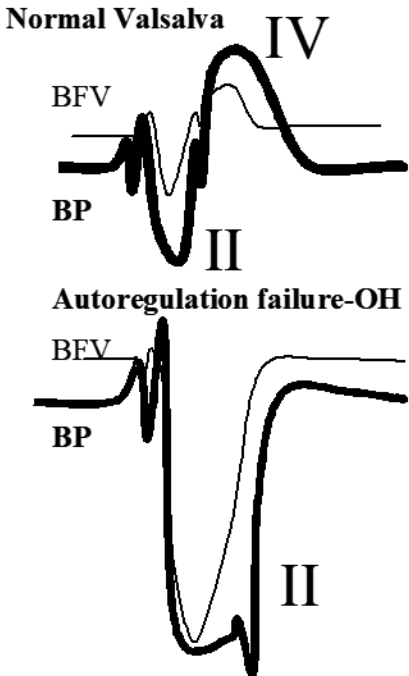
The goal was to understand the relationship between changes of blood pressure and cerebral blood flow upon standing-up to determine if the cerebral autoregulation was impaired in the patients with diabetic autonomic neuropathy. We hypothesized that patients with diabetic autonomic neuropathy with OH may be at risk for cerebral hypoperfusion if blood pressure falls below autoregulated range.

End Points:

1. The change of cerebral blood flow velocity between baseline and the sit-to-stand test and Valsalva maneuver.
2. The index of autoregulation.
3. The relationship between blood pressure and BFV based on the transfer function of power spectrum.

Blood Flow Velocities during Sit-to-Stand: We measured the change in mean blood pressure (MBP) between sitting and standing-up (specifically between the 5-min average MBP and the minimum MBP upon standing-up), and the change of cerebral blood flow (specifically between the 5-min average BFV) while sitting and BFV value corresponding to BP minimum were recorded.

Blood Flow Velocities during Valsalva maneuver: Figure 2 illustrates BFV/BP profile during Valsalva maneuver with autoregulation vs. autoregulation failure. We measured minimum MBP during phase II of Valsalva maneuver and maximum MBP during phase IV and the change of MBP between the baseline and phase II and phase IV. Corresponding values of BFV were computed.



Autoregulation-Regression Analysis: MBP/BFV relationship were determined using regression analysis that was a robust method for evaluation of autoregulation (14) (15). Our tilt studies with OH patients had shown (16, 17) that the coefficient of determination (R^2) could identify autoregulation failure (positive correlation) and that the slope of regression provided an index of severity of such failure. BFV/BP correlation (18) could be used to mark autoregulation limits. Lack of BFV/BP correlation suggested normal autoregulation (see preliminary results).

Index of autoregulation proposed by Tiecks et al 1995 (19) Briefly, this model uses 2nd order linear differential equations to define beat-to-beat BFV/BP relationship (19). The autoregulatory index (ARI) consists of three parameters: 1) the time constant; 2) damping factor; and 3) dynamic gain. These indices were incorporated in estimates of autoregulatory gain (0 = no autoregulation and 9 = perfect autoregulation), including MBP and estimated cerebral perfusion pressure. The validity of this model for our patient population were evaluated.

Power Spectrum and Transfer Function: We used time-frequency mapping that will provide dynamic index of BFV/BP relationship and transfer function during nonstationary transitions (16, 20-23). BFV oscillations play a role in the dynamic redistribution of cerebral blood volume. They resulted from interaction between BP, intracranial pressure and vasomotor tone and are good candidates for evaluation of neurogenic autoregulation. In the OH patients, both MBP and BFV oscillations were diminished and ARI was altered (24-26). Transfer function was computed from BP and BFV waveforms resampled at 50 Hz.

Analysis: Primary objective of Aim 2 was to compare the differences in BFV before and during the sit-to-stand test for the three groups. Data were summarized for each patient and group using descriptive statistics. One-way ANOVA was used initially for group comparisons. Due to possible confounding by other variables, linear regression models was applied for data analysis. BFV was the dependent variable and group was the independent variable. Covariates to be included in the model are DM duration, OH vs. No-OH, MBP change during sit-to-stand, age, gender, and race. Time frequency indices such as spectral power, frequency and transfer function in the each range of interest were derived for each variable during supine rest and tilt.

Power for Specific Aim #2:

Assuming a standard deviation of 3.8 cm/s for the difference in BFV for two groups (8), we would have 90% power to detect a difference of 4 cm/s between the DM-OH+ group vs. control or between DM-OH- group and $\alpha=0.05$.

Statistical Analysis for the Specific Aim 3:

The goal was to determine relationship between WMC and cerebral vasoregulation, and between WMC and orthostatic hypotension. Cerebral vasoregulation was measured using TCD under the specific Aim 1.

End Points:

1. The WMC distribution and volume in diabetic autonomic neuropathy with OH.
2. The relationship between WMC and impaired vasoreactivity.
3. The relationship between WMC and orthostatic hypotension.

WMC distribution and volume: We measured WMC distribution and volume, using T2 weighted FLAIR and FSE images at 3 Tesla MRI. WMC were ill-defined and moderately hypodense areas of > 5 mm on T2 weighted images. Lacunes were described as well-defined areas of > 2 mm with the signal characteristics same as cerebrospinal fluid. Lesions < 2 mm were considered as perivascular spaces, except around the anterior commissure, where perivascular spaces could be enlarged. T1-weighted gradient echo images were used for vessel identification (27). WMC were identified on both FLAIR and FSE images. Changes in basal ganglia were rated the same way and considered as WMC, if located in the gray matter.

1) First, all images were visually inspected and scored using a visual rating scale from 0 to 3: 0=no lesions (including symmetrical, well defined caps or bands), 1=focal; 2=beginning confluence; 3=diffuse involvement of the entire region. Rating score for basal ganglia: 0=no lesions; 1=1 focal lesion (>5mm); 2=>1 focal lesion; 3=confluent lesions. WMC distribution were qualitatively determined in the anterior, middle and posterior cerebral arteries distribution in each hemisphere as WMC present vs. absent. WMC volume was calculated using image segmentation method (28).

WMC and cerebral vasoregulation Relationship between WMC and cerebral vasomotor range were evaluated using regression modeling. WMC (distribution & volume) were an independent variable and vasomotor range as a dependent variable (calculation of vasomotor range is described under the Aim 1).

WMC and orthostatic hypotension Relationship between WMC and OH were evaluated using regression modeling with WMC (volume) as independent variable and change of MBP between supine rest and tilt-up as a dependent variable.

Power for Specific Aim #3:

Based on the published Rotterdam population studies we expected to find significant correlations between impaired cerebral vasoreactivity and WMC volume (29). Our sample size provided us with 99% power to detect differences between WMC volume (0.6 ± 1.0 ml) in patients with abnormal vasoregulation (VMR < 2.7 %/mm Hg) vs. WMC volume (mean 0.16 ml) in patients with normal vasoregulation based of standard deviation 1.0. We also identified the patients with mild WMC abnormalities with WMC volume around 0.4 ml with 90% power.

After a graphical inspection of the data, followed by an additional screen for potential outliers and data errors, and assessment of distributional assumptions, standard descriptive statistics (e.g., mean and standard deviation, median and interquartile range) were calculated for each group separately. Univariate comparisons between groups used standard methods that take into account the matching (e.g. repeated measures ANOVA for continuous normally distributed

data, Kruskal-Wallis test for other continuous data, Chi-square test for categorical data). Modeling was done in the generalized linear model framework, which incorporated logistic regression as a special case. All modeling took into account the individual matching of subjects across the three groups. Modeling of presence (volume and distribution) of white matter changes (as the outcome variable) incorporated known risk factors (e.g. age, DM, retinopathy), and potential confounders identified during the univariate analyses of factors with patient group and with presence/extent of white matter changes. We used the effect of condition on cerebral blood flow, perfusion and vasomotor reactivity as predictors and test whether patient group (DM, presence of OH) was predictive of outcome after adjustment for these factors.

SUBJECT SELECTION

Subject selection criteria in this section details the methods, criteria, and procedures in place when subjects were still being enrolled in this study. Please note that this study is closed for data analysis only.

Inclusion criteria:

Diabetes with OH: 40 patients with type 1 and type 2 diabetes for > 1 year who met the criteria for diabetic autonomic neuropathy with OH (BP drop >20/10 mm Hg) with autonomic symptoms score 5-10 1-3,30,31, age 50-75 years were recruited. Patients with diabetic autonomic neuropathy were matched with 40 diabetic patients without OH by DM duration and age + 5 years.

Diabetes without OH: 40 diabetic patients without OH (average BP change <10/5 mm Hg over 3 minutes), autonomic symptom score 0-1 were matched with DM-OH+ group.

Controls: 40 age-matched + 5 years healthy subjects with no history of DM or OH served as controls and were matched with diabetes groups by age + 5 years.

Exclusion criteria:

All conditions that could affect the subjects' ability to cooperate including: dementia, epilepsy, alcohol and other drug abuse. OH due to other causes than DM, malignant neoplasms, renal, liver or congestive heart failure, significant nephropathy, kidney or liver transplant, uncontrolled hypertension with BP>200/110 mmHg, clinically significant arrhythmias, documented carotid artery stenosis >50% documented by Doppler ultrasound. MRI-exclusion criteria: The subjects with metallic bioimplants, claustrophobia, inability to cooperate or morbid obesity BMI >40. TCD exclusion criterion was an inability to obtain TCD signal due to poor insonation window.

Recruitment/screening:

Diabetic patients were recruited from Joslin Diabetes Clinic and from the Harvard Cooperative Program on Aging (HCPA) research subject registry. Joslin Diabetes Center has database of ≈15,000 diabetic patients and HCPA has a registry of > 5000 older subjects in the Boston area. The Investigator or co-investigators explained the purpose of this study to the patient prior to entering the patient in the study. The patients were asked to sign informed consent and were provided with a copy of the consent form.