



Published in final edited form as:

*Neuroepidemiology*. 2015 ; 45(1): 1–11. doi:10.1159/000435775.

## Relationships between cognitive performance, neuroimaging, and vascular disease: the DHS-Mind Study

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### Abstract

**Background**—Type 2 diabetes mellitus increases risk for cognitive decline and dementia; elevated burdens of vascular disease are hypothesized to contribute to this risk. These relationships were examined in the Diabetes Heart Study-Mind using a battery of cognitive tests, neuroimaging measures, and subclinical cardiovascular disease (CVD) burden assessed by

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#### Author Contributions

F.-C.H analyzed data and wrote the manuscript, LMR contributed to the statistical analysis and reviewed and edited the manuscript, CEH collected, managed data, reviewed and edited the manuscript, AC reviewed and edited the manuscript, JX collected and managed data, JJC initially designed the Diabetes Heart Study and ascertained and clinically evaluated patients, BIF initially designed the Diabetes Heart Study, ascertained and clinically evaluated patients, and reviewed and edited the manuscript, JAM collected the neuroimaging data, and reviewed and edited the manuscript, JDW collected the cognitive performance data, DWB led the Diabetes Heart Study and reviewed and edited the manuscript. FCH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

#### Conflicts of Interest

The authors declare no conflicts of interest.

coronary artery calcified plaque (CAC). We hypothesized that CAC would attenuate the association between neuroimaging measures and cognition performance.

**Methods**—Associations were examined using marginal models in this family-based cohort of 572 European Americans from 263 families. All models were adjusted for age, gender, education, type 2 diabetes, and hypertension, with some neuroimaging measures additionally adjusted for intracranial volume.

**Results**—Higher total brain volume (TBV) was associated with better performance on the Digit Symbol Substitution Task (DSST) and Semantic Fluency (both  $p = 7.0 \times 10^{-4}$ ). Higher gray matter volume (GMV) was associated with better performance on the Modified Mini-Mental State Examination and Semantic Fluency (both  $p = 9.0 \times 10^{-4}$ ). Adjusting for CAC caused minimal changes to the results.

**Conclusions**—Relationships exist between neuroimaging measures and cognitive performance in a type 2 diabetes-enriched European American cohort. Associations were minimally attenuated after adjusting for subclinical CVD. Additional work is needed to understand how subclinical CVD burden interacts with other factors and impacts relationships between neuroimaging and cognitive testing measures.

### Keywords

Cognitive testing; Neuroimaging; Coronary artery calcified plaque; Type 2 diabetes; Vascular disease

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## INTRODUCTION

Type 2 diabetes mellitus is associated with acceleration of mild age-related declines in a variety of cognitive domains and increased risk of overt dementia (1). Similarly, changes in the brain in individuals with type 2 diabetes, assessed using magnetic resonance imaging (MRI) derived neuroimaging measures, including increased white matter lesion volume, reduced brain volume, and reduced white matter fractional anisotropy have been reported (2–5). Cardiovascular disease (CVD) is significantly elevated in people with type 2 diabetes. Both clinical and subclinical CVD have been hypothesized as causal factors in the increased risk of dementia and cognitive decline in people with type 2 diabetes. CVD may be associated with both neuroimaging measures and cognitive tests and may influence relationships between these measures. Vascular calcified plaque, frequently measured by computed tomography as coronary artery calcified plaque (CAC), is a well-documented independent predictor of CVD events and mortality (6–12) in people with and without diabetes. CAC is a measure of vascular disease burden; individuals affected by type 2 diabetes tend to have higher vascular calcified plaque than non-diabetic controls (13).

The Diabetes Heart Study (DHS)-Mind is a single-center, family-based study which assessed measures of cognitive performance, neuroimaging, and subclinical CVD in a European American population enriched for type 2 diabetes. This study population was chosen based on the high burden of CAC in participants. Our prior work found that CAC attenuates the association between cognition and type 2 diabetes in tasks that are associated with type 2 diabetes and is associated with cognitive performance on other tests (e.g., Rey

Auditory-Verbal Learning Task (RAVLT)) independently of T2D (14). Here, associations between tests of multiple cognitive domains, neuroimaging measures, and CAC were examined. We assessed whether CAC mediated the association between cognitive performance and neuroimaging measures; we hypothesized that CAC would attenuate these relationships.

## MATERIALS AND METHODS

### Study Design and Sample

Participants included individuals enrolled in the DHS, designed to identify genetic and epidemiological factors contributing to CVD risk in type 2 diabetes (15,16). Participants were siblings concordant for type 2 diabetes without advanced renal insufficiency (baseline serum creatinine concentration <2.0 mg/dl), and where possible, their unaffected siblings. Participants were recruited from internal medicine and endocrinology clinics and the community from 1998 through 2006 in western North Carolina. Ascertainment and recruitment have been described in detail (15–18). Type 2 diabetes-affected participants had diabetes diagnosed after the age of 35 years treated with changes in diet and exercise and/or insulin or oral agents in the absence of a history of ketoacidosis or initial treatment solely with insulin. Diabetes diagnosis was confirmed by measurement of fasting glucose and glycated hemoglobin (Hb) A<sub>1C</sub> at the exam visit. Extensive measurements of CVD risk factors, including computed tomography (CT)-based measures of vascular calcified plaque, were obtained during baseline exams from 1998–2006.

The DHS-Mind is an ancillary study to the DHS. It was initiated in 2008 to perform cognitive testing and neuroimaging to investigate risk factors for cognitive decline in the cohort enriched for type 2 diabetes. Participants from the original DHS investigation were re-examined on average  $6.7 \pm 1.6$  years after their initial visit. Participant examinations were conducted in the General Clinical Research Center of the Wake Forest Baptist Medical Center. A total of 893 European American participants were recruited; 572 participants returning from the initial DHS and 321 newly examined. Returning participants have data from the baseline exam, including measures of subclinical CVD (n=572). The 321 newly recruited participants generally have neuroimaging and cognitive test data, but not CT-based vascular imaging. Depression was measured in 429 (of 572) participants using the Center for Epidemiologic Studies Depression (CES-D) Scale 10 item measure, considered to have high sensitivity and specificity in patients with diabetes (19). Study protocols were approved by the Institutional Review Board at Wake Forest School of Medicine. All participants provided written informed consent prior to participation.

### Coronary Artery Calcified Plaque

Subclinical CVD was assessed by measurement of calcified plaque in the coronary (CAC) vascular bed with calcium scores calculated as described (16). Briefly, all cardiac CT examinations were performed on a single-slice helical CT or a four-channel multidetector CT with cardiac gating and capable of 500 ms temporal resolution (HiSpeed LX and LightSpeed QXi with the SmartScore Cardiac scan package; General Electric Medical Systems, Waukesha, WI). After a scout image of the chest, the heart was imaged during

suspended respiration at end inspiration. Scan parameters were 3 mm slice thickness, 26 cm display field of view, retrospective cardiac gating, 120 KV, 240 mA, and CT scan pitch adjusted to heart rate for the single-slice system and 2.5 mm slice thickness in four-slice mode, 26 cm display field of view, prospective cardiac gating at 50% of the RR interval, 120 KV, and 240 mA for the multidetector CT.

### Cognitive Testing

Participants were administered a battery of cognitive tests as previously described (14). Global cognitive function was assessed with the Modified Mini-Mental State Examination (3MSE), a measure used clinically to assess dementia with scores ranging from 0–100 (20). The Digit Symbol Substitution Task (DSST) was used as a measure of processing speed and to a lesser extent, working memory, reported here as the number of correct responses within 2 minutes (21). The Stroop Task (Stroop) was used to test executive function and was reported here as the difference in response times between subtest 2 and subtest 3 (22). Verbal memory was tested with the RAVLT, a 15-item word-list recall task (23). Here we reported the total number of words recalled across the first five trials. Phonemic Fluency was measured via the Controlled Oral Word Association Task (COWA), reported here as the sum of words generated during 1 minute for three different letters (F, A, S). Semantic Fluency was measured by asking participants to generate words related to two different categories (kitchen, animals) for 1 minute, and the sum of these is used for analysis (24,25).

Participants were not excluded for 3MSE scores or other indices of cognitive function indicative of mild cognitive impairment or dementia. Forty-two participants were part of a pilot feasibility study and did not complete the Phonemic or Semantic Fluency tasks. Seven participants who reported being color-blind or missed a significant number of questions on the color-naming portion of the task were excluded from the Stroop task. DSST scores were missing for three participants due to problems with writing (n=1) and vision (n=2). The RAVLT was not completed by two participants and 3MSE was not completed by one participant. For the 3MSE, DSST, RAVLT, and Phonemic and Semantic Fluency, higher scores indicate better cognitive performance; for the Stroop Task lower scores indicate better performance.

### Neuroimaging

**Magnetic resonance (MR) image acquisition**—MR imaging was performed on a 1.5-T GE EXCITE HD scanner with twin-speed gradients using a neurovascular head coil (GE Healthcare, Milwaukee, WI). A 3D volumetric Inversion Recovery SPGR sequence was used to obtain high-resolution T1 anatomic images (TR=7.36 ms; TE=2.02 ms; TI=600 ms; FA=20 degrees; 124 slices, FOV=24 cm, matrix size = 256×256, 1.5 mm slice thickness). Fluid-attenuated inversion recovery (FLAIR) images were acquired in the axial plane (TR=8002 ms; TE=101.29 ms; TI=2000 ms; FA=90 degrees; FOV=24 cm; matrix size = 256 × 256; 3-mm slice thickness). Echo-planar imaging with 25 directions was used to perform whole brain diffusion tensor imaging (DTI) (TR=16000; TE=84.9; FA=90; b value = 0/1000, FOV = 280 cm, matrix size = 256×256, 3 mm slice thickness). A Q2TIPS-FAIR sequence as previously described was used to generate quantitative cerebral blood flow maps (26). This sequence generates 60 tag and control image pairs. Imaging parameters are :

echo time 28ms,  $TI_1$ -800ms,  $TI_1s$  1200ms,  $TI$  2000ms, TR 3000ms, receiver bandwidth 62.5 kHz, flip angle 90 degrees, field of view 24 cm (frequency) x 18 cm (phase), an acquisition matrix 64x48 (11 slices, 8 mm thickness, 0mm slice gap), and frequency encoding direction anterior/posterior. A bipolar diffusion gradient with an equivalent b value of 5.25 mm<sup>2</sup>/sec was added to suppress intra-arterial spins (27).

**Image segmentation**—Structural T1 images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), normalized to Montreal Neurologic Imaging (MNI) space, and modulated with the Jacobian determinants (non-linear components only) of the warping procedure to generate volumetric tissue maps using the Dartel high-dimensional warping and the SPM8 (28) new segment procedure as implemented in the VBM8 toolbox (<http://dbm.neuro.uni-jena.de/vbm.html>). This modulation step did not include the affine component of the normalization parameters, thereby correcting the volumetric tissue maps for total intracranial volume (ICV). ICV (GMV + WMV + CSF), total brain volume (TBV) (GMV + WMV), GM volume (GMV), and WM volume (WMV) were determined from the VBM8 automated segmentation procedure, which outputs values for native space total GM, WM, and CSF volumes. The normalized GM and WM segmentation maps (without modulation) were binarized at a probability threshold of 0.5 to create segmentation masks for use in generating the tissue-specific measures of diffusion and cerebral blood flow.

**Diffusion tensor processing**—FSL was used to perform diffusion tensor pre-processing (29). Eddy current correction of the diffusion tensor images was performed using FSL `dti_eddy` by normalizing each image to the baseline (B0) image using the mutual information registration algorithm. The Camino software package was used to compute the diffusion tensor ([www.camino.org.uk](http://www.camino.org.uk)). The resulting tensor images were converted to NIfTI symmetric positive orientation using the Diffusion Tensor Imaging ToolKit (DTI-TK) (<http://www.nitrc.org/projects/dtitk>). DTI scalar metrics, including fractional anisotropy (FA) and mean diffusivity (MD), were computed using DTI-TK. DTI scalars were normalized to MNI space by coregistering the MD image to the T1 structural data using SPM8 and then combining this transformation matrix with the parameters computed in the VBM8 normalization procedure. The global MD and FA measures analyzed here (WMMD, GMMD, WMFA, GMFA) were calculated based on a white matter segmentation mask generated from the VBM8 segmentation procedure from the structural T1 images applied to the DTI scalar images.

**Cerebral blood flow processing**—Perfusion images were generated using a previously described fully automated data processing pipeline (30). Quantitative data processing includes data cleaning (removal of individual images with noise spikes, poor tissue segmentation, or severe motion artifact), realignment (separately for label and control images), spatial smoothing, and calculation of mean cerebral blood flow (CBF) maps; our methods and experience with this technique has been well documented (30–32). The CBF maps were normalized to MNI space by coregistering to the T1 structural data using SPM8 and then combining this transformation matrix with the parameters computed in the VBM8 normalization procedure, allowing derivation of the gray matter cerebral blood flow

(GMCBF) measure analyzed. Two participants with CBF below 7.9 mL/100g of tissue/min (biological limit) were removed (33).

**White Matter Lesion (WML) Segmentation**—WML segmentation was performed using the lesion segmentation toolbox (LST) (34) for SPM8 at a threshold ( $k$ ) of 0.25. We previously validated the LST for use in the DHS-Mind in a sample of 100 participants against expert manual segmentation, as well as identifying the optimum threshold in this population (35). Normalization to MNI space was accomplished by coregistration with the structural T1 and applying the normalization parameters computed in the VBM8 segmentation procedure. The total white matter lesion volume (WMLV) measure used in these analyses was determined by summing the binary lesion maps and multiplying by the voxel volume. Neuroimaging data for some participants could not be acquired for a number of reasons and contra-indications to MRI: claustrophobia ( $n=27$ ), large body size ( $n=14$ ), non-MRI compatible implant ( $n=1$ ), pacemaker ( $n=17$ ), stent (6), metal in the body (e.g., earrings;  $n=10$ ), and miscellaneous (e.g., tissue expander, etc;  $n=9$ ).

### Statistical Analyses

Statistical analysis was completed using SAS software version 9.3 (SAS Institute, Cary, NC, USA). Summary statistics such as means, standard deviations (SD), medians, and ranges were computed for the continuous characteristics, and percentages were computed for discrete characteristics. Imaging variables and cognitive testing data were collected in the DHS-Mind sample. However, calcified plaque variables were only available for the 572 participants in the DHS baseline exam. The primary analysis, studying the mediation effect of CAC, was conducted using the subset with CT data collected at the baseline exam. To assess associations between cognitive testing and imaging variables, the entire DHS-Mind sample was used to maximize power (Supplemental Table S2).

Stroop, CAC, TBV, WMLV, and WMV were log transformed and GMCBF were square root transformed to approximate the normality assumption. Imaging variables and CAC were standardized for relative importance comparison, allowing assessment of which variable was more highly associated with cognitive testing data. The following associations were studied using the marginal models incorporating generalized estimating equations (GEE) approaches: Model 1 explores associations of cognitive testing on neuroimaging variables; Model 2 explores associations of cognitive testing on CAC; Model 3 explores associations of neuroimaging variables on CAC; and Model 4 explores associations of cognitive testing on neuroimaging variables after adjusting for CAC. Imaging variables were adjusted in Models 1 and 4 one at a time. Cognitive testing data were treated as the dependent variables in Models 1, 2, and 4 and were not standardized for ease of interpretation. Imaging variables were treated as the dependent variables in Model 3. The comparison between Model 1 and Model 4 was our primary focus. However, we further examined Models 2 and 3 in this type 2 diabetes-enriched European American cohort. Marginal models account for familial correlation using a sandwich estimator of the variance under exchangeable correlation. All models were adjusted for age, gender, educational attainment (not adjusted when exploring the association between neuroimaging and CAC), type 2 diabetes status, and hypertension, with models examining TBV, WMLV, GMV, and

WMV additionally adjusted for ICV. A Bonferroni correction was used to correct for multiple comparisons.

The following sensitivity analyses were performed. First, because depression may affect the performance on cognitive testing, the association analyses (relating imaging measures to cognitive testing (Model 1); relating CAC to cognitive testing (Model 2); relating CAC, imaging to cognitive testing (Model 4)) were further adjusted for depression score. This analysis was treated as a sensitivity analysis, because only 75% of the sample had data on depression scores. Secondly, more than 80% of the sample had type 2 diabetes. Whether the association between neuroimaging and cognitive testing in the diabetes group was the same as that in the non-diabetes group was assessed by performing the analysis (Model 4) stratified by diabetes status.

## RESULTS

The DHS collected data on CVD risk factors from 1998–2006, including measures of vascular calcified plaque. The DHS-Mind subsequently collected cognitive testing and neuroimaging data from 893 European Americans from 2008–2013, including 572 from the original DHS sample. The demographic characteristics for the entire DHS-Mind sample (n=893) and the subset from the baseline exam (n=572) are shown in Table 1. With the exception of measures of lipids and CAC, all data is from the DHS-Mind visit. Demographic factors, cognitive performance, and imaging measures for the DHS baseline subset were broadly similar to those for the whole sample. The baseline DHS sample was slightly older (1.5 years) and had slightly lower type 2 diabetes prevalence (80.9%) and educational attainment (28.3% greater than high school). In addition, the baseline sample tended to use less anti-diabetic medication (67.1% vs. 73.2%) but more cholesterol-lowering (53.7% vs. 48.3%) and anti-hypertensive medication (73.1% vs. 67.4%).

Cognitive testing demonstrated substantial heterogeneity in performance; scores ranged from levels indicative of mild dementia (3MSE<77) to above average. Overall, mean and median scores on cognitive tests were slightly lower than expected in the general population. The baseline sample had lower scores on the 3MSE, DSST, Phonemic and Semantic Fluency, but higher Stroop, compared to the entire DHS-Mind sample, indicative of slightly poorer cognitive testing performance in the baseline sample. Furthermore, the baseline sample had lower TBV, GMV, and ICV, but had higher WMFA, GMFA, and GMCBF compared to the entire DHS-Mind sample.

### Association of Cognitive Testing and Neuroimaging Variables (Model 1)

The association between cognitive testing and imaging variables (Table 2) included only individuals from the baseline samples. Analyses were adjusted for age, gender, educational attainment, type 2 diabetes, and hypertension, with TBV, WMLV, GMV, and WMV additionally adjusted for ICV. Correcting for multiple comparisons, significance was accepted for alpha = 0.0014.

TBV (the sum of GMV and WMV) was positively associated with DSST ( $p=2.4 \times 10^{-4}$ ) and Semantic Fluency ( $p=7.0 \times 10^{-4}$ ), meaning that greater brain volume was associated with

better processing speed performance. Per SD increase of TBV, DSST would increase by 9.89 correct responses (equivalent to 18.9 years of aging effect in the opposite direction) and Semantic Fluency would increase by 5.00 named items (equivalent to 24.0 years of aging effect in the opposite direction). GMV was associated with performance on the 3MSE ( $p=7.0 \times 10^{-4}$ ) and Semantic Fluency ( $p=9.0 \times 10^{-4}$ ). Per SD increase of GMV, 3MSE would increase 2.86 points (equivalent to 19.9 years of aging effect in the opposite direction) and Semantic Fluency would increase by 2.46 named items (equivalent to 11.8 years of aging effect in the opposite direction). WMV was not significantly associated with any cognitive test.

WM integrity was assessed using WMLV segmentation from the FLAIR images and FA from DTI analysis, which can detect subclinical changes in WM microstructure. Both WMLV ( $p=1.0 \times 10^{-4}$ ) and WMFA ( $p=8.0 \times 10^{-6}$ ) were associated with DSST. As in previous studies, greater WMLV was associated with poorer scores on the DSST ( $\beta = -2.55$ ). Increased FA, a marker of white matter health, was associated with better scores on the DSST ( $\beta=3.26$ ). Higher WMFA was also associated with better performance on the Stroop test ( $\beta$  estimate  $=-0.09$ ,  $p=4.6 \times 10^{-4}$ ). Note that in this study WMFA was included in our main analysis, as this is the most commonly analyzed DTI measure, with global WMFA previously associated with diabetes status (4). The analyses for WMMD, GMMD, and GMFA are presented in Supplemental Table S1 for reference.

Supplemental Table S2 summarizes associations between cognitive testing and standardized imaging measures using the entire DHS-Mind sample. As in the subsample with CAC data, TBV was positively associated with DSST ( $p=6.0 \times 10^{-6}$ ) and Semantic Fluency ( $p=6.0 \times 10^{-6}$ ). In this larger sample, TBV was also positively associated with performance on the RAVLT ( $p=6.1 \times 10^{-5}$ ) and Phonemic Fluency ( $p=1.2 \times 10^{-4}$ ). GMV was again positively associated with 3MSE score ( $p=7.3 \times 10^{-5}$ ) and Semantic Fluency ( $p=8.5 \times 10^{-5}$ ), and, in the larger sample, with DSST performance ( $p=2.0 \times 10^{-6}$ ). As in the smaller sample, WMV was not associated with any cognitive measures. WMLV was again negatively associated with DSST score ( $p=2.2 \times 10^{-7}$ ), and in the larger sample, with RAVLT ( $p=9.9 \times 10^{-5}$ ), Phonemic Fluency ( $p=6.8 \times 10^{-4}$ ) and Semantic Fluency ( $p=2.0 \times 10^{-6}$ ). WMFA was positively associated with only RAVLT ( $p=4.5 \times 10^{-4}$ ). Thus analyses of the entire sample ( $n=893$ ) largely replicate results from the subset with data for CAC, and, in many cases, the associations are statistically stronger.

### **Association of Cognitive Testing and Neuroimaging with Vascular Calcified Plaque (Models 2 and 3)**

The associations between cognitive testing measures and standardized log transformed CAC are presented in Table 3. After adjusting for age, gender, educational attainment, type 2 diabetes, and hypertension and correcting for multiple comparisons ( $\alpha=0.0083$ ), only RAVLT performance was negatively associated with log transformed CAC ( $p=0.003$ ). That is, higher log transformed CAC burden was associated with lower word recall, such that 1.27 fewer words (equivalent to 2.9 years of aging effect in the opposite direction) were recalled for each SD increase of log transformed CAC. Performance on the DSST and Semantic Fluency were nominally associated ( $p<0.05$ ) with CAC burden.



Associations between standardized imaging measures and log transformed CAC are shown in Table 3, and associations with secondary imaging outcomes are presented in Supplemental Table S3. Analyses were adjusted for age, gender, type 2 diabetes, hypertension, and except for FA, adjusted for ICV. After correcting for multiple comparisons ( $\alpha=0.0083$ ), TBV (beta estimate=-0.05,  $p=6.0 \times 10^{-6}$ ) and GMV (beta estimate=-0.08,  $p=7.0 \times 10^{-4}$ ) were negatively associated with CAC. That is, higher CAC burden was associated with lower brain volumes.

#### **Association of Cognitive Performance and Imaging Variables after Adjusting for Vascular Calcified Plaque (Model 4)**

Next, analyses were performed to assess whether CAC mediates the relationship between cognitive performance and neuroimaging measures. The associations between imaging measures and cognitive testing measures following adjustment for log transformed CAC (Table 4) were very similar to those without the additional CAC adjustment (Table 2). After adjusting for CAC burden, TBV remained positively associated with DSST (beta estimate=10.01,  $p=4.5 \times 10^{-4}$ ) and Semantic Fluency (beta estimate=5.07,  $p=0.001$ ) with little change in effect sizes or p-values. GMV was still significantly associated with 3MSE (beta estimate=2.98,  $p=9.3 \times 10^{-4}$ ); however, the association between GMV and Semantic Fluency dropped below multiple corrections threshold significance (beta estimate = 2.16,  $p=0.004$ ). The association between WMLV and DSST was slightly strengthened by adjustment for CAC (beta estimate=-2.71,  $p=5.1 \times 10^{-5}$ ), and the associations between WMFA and DSST (beta estimate=3.20,  $p=1.3 \times 10^{-5}$ ) and the Stroop test (beta estimate=-0.09,  $p=9.8 \times 10^{-4}$ ) remained significant. In addition, the association between WMFA and Semantic Fluency was strengthened (beta estimate=1.48,  $p=8.7 \times 10^{-4}$ ). Associations with secondary imaging outcomes are presented in Supplemental Table S4.

When comparing the regression coefficients between the models with and without CAC adjustment, only the coefficient between GMV and Semantic Fluency changed over 10%. The regression coefficient changed from 2.46 to 2.16 after adjusting for CAC (12% reduction) and the p-value became non-significant. These results suggest that CAC may not be a significant mediator for the associations between imaging and cognitive testing in the DHS-Mind cohort. However, it may mediate the association between GMV and Semantic Fluency slightly.

#### **Sensitivity analysis**

To demonstrate the robustness of our results, we performed two sensitivity analyses. First, the association analysis further adjusted for CES-D depression scores showed similar results (data not shown). Second, the analyses stratified by diabetes status (Model 4) are presented in Supplemental Table 5A and 5B. For the diabetes-only analysis, the results were similar to previous results in the full sample. Due to the smaller sample size ( $n=463$ ), results were somewhat less significant. Previously significant associations with DSST and the association between TBV and Semantic Fluency remained. In the non-diabetic sample ( $n=109$ ), the only significant result was observed for association between WMFA and DSST.

## DISCUSSION

Previous analysis of the DHS-Mind cohort, a type 2 diabetes enriched study population, indicated that higher levels of subclinical CVD were associated with poorer performance on the DSST, RAVLT, and Semantic Fluency (14). Here, we investigated whether cognitive function in this cohort was associated with brain imaging outcomes, and whether neuroimaging-cognition relationships were influenced by burden of subclinical CVD. In the subsample where complete data on cognition, imaging, and subclinical CVD were available, imaging variables were associated with the 3MSE, DSST, Stroop, and Semantic Fluency. The associations between imaging and cognitive testing measures changed little after adjusting for CAC. Semantic Fluency was the only cognitive task that showed significant changes in association after adjusting for CAC. The effect size for association between GMV and Semantic Fluency was reduced by 12% and p-value dropped to non-significance, suggesting the relationship between GMV and Semantic Fluency may be mediated by subclinical CVD. In addition, the association between WMFA and Semantic Fluency strengthened slightly to reach statistical significance after accounting for the variance explained by CAC.

These results indicate that both brain imaging measures and subclinical CVD are associated with cognitive performance in a European American cohort with a high prevalence of type 2 diabetes. However, for global cognition (assessed via 3MSE) and processing speed (assessed via DSST), these cognition-neuroimaging associations are not explained by the burden of subclinical CVD (CAC). However, CAC may mediate the association between neuroimaging variables (GMV and WMFA) and Semantic Fluency. While not statistically significant, there was a trend ( $p=0.014$ ) for association between the GMV and RAVLT, and the magnitude of this trend was decreased by 14% after adjusting for CAC, similar to the effects on Semantic Fluency. Our findings are in concordance with two other studies investigating the relationships between CAC, cognition, and neuroimaging measures, although they examined the mediation effect of brain imaging measures in the relationship between CAC and cognitive status instead (36,37). Vidal and colleagues reported participants with increasing CAC were more likely to have dementia and poorer cognitive performance; these relationships were mediated by brain volumes and lesions (36). Rosano et al. (37) reported that the presence of any MRI abnormalities, including ventricular enlargement, white matter hyperintensities, and subcortical brain infarcts, attenuated the association between CAC and abnormal cognitive status in older adults.

There are differences in the significant associations reported here and our previous report in this cohort. We previously reported that CAC was associated with poorer performance on the RAVLT, DSST, and Semantic Fluency, and that CAC attenuated the association of type 2 diabetes with performance on these cognitive tests (14). The main reason for these differences in association between cognition and CAC is that in this analysis, we applied a correction for multiple comparisons and also the sample size is slightly larger with more data being collected. The previous associations with the RAVLT and DSST were observed, but did not meet the more stringent correction for multiple comparisons. Furthermore, it is worth noting that our sister study with the same study design but recruiting only African American participants (AA-DHS MIND) did not observe any association between CAC and

cognitive testing measures (Barry Freedman, personal communication). The mechanism for the racial difference requires further research.

There is an increasing body of literature reporting association of CVD risk factors with brain structure and function (36–38). CAC has been shown to negatively associate with TBV, GMV, WMV, and positively associate with WMLV (36). We observed the associations with TBV and GMV, but not with WMLV and WMV. Differences in neuroimaging methods and sample ascertainment, notably enrichment for type 2 diabetes in the DHS, may partially explain these discrepancies. No prior studies reported the associations with WMFA and GMCBF; these measures have not been analyzed frequently, however.

Neuroimaging measures such as higher WMLV and lower GMV and WMV have been associated previously with lower scores on cognitive tests, including tests of memory, processing speed, and executive function, in individuals with and without type 2 diabetes and in both cross-sectional and longitudinal studies (39–46). Factors such as blood pressure and glycemic control may influence these associations. Jennings et al. (47) found that degree of lowering blood pressure affected the association between cognitive performance and patterns of regional CBF. The ACCORD MIND study (48) showed that there was a significant glucose treatment effect on TBV, but no significant effects on cognitive outcomes. Midlife systolic blood pressure has been found to predict both cognitive function and brain imaging (49). Diabetes increases risk for cognitive decline and changes in cerebral WM (44). In our type 2 diabetes-enriched study population (16.6 year mean diabetes duration), we similarly showed that TBV, WMLV, and GMV were associated with cognitive testing including 3MSE, DSST, and Semantic Fluency. In addition, WMFA was associated with DSST and Stroop in the baseline sample and with RAVLT in the whole sample. It has been shown that WMFA is associated with cognitive function in the elderly (50).

For the diabetes-only analysis, the results were similar to previous results in the full sample. Due to the smaller sample size ( $n=463$ ), results were less significant. However, the associations still reached the nominal significance level at  $p<0.05$ , because more than 80% of the study population had diabetes and the previous analysis appears to have been mainly driven by this majority. In addition, non-diabetic participants likely share genetic and environmental effects (and had similar demographic characteristics) as their diabetic siblings. In the smaller non-diabetic analysis ( $n=109$ ), the only significant result was observed for association between WMFA and DSST. The association of Semantic Fluency on TBV and WMFA did not reach nominal significance. This analysis suggests that the association of Semantic Fluency on neuroimaging variables may differ between diabetic and non-diabetic subjects, but requires further study.

This study has several strengths. Prior studies often evaluated small cohorts and lacked analysis of multiple neuroimaging and cognitive testing measures. The DHS sample provides a foundation for understanding the potential importance of early intervention to prevent the cognitive decline and devastating brain complications of type 2 diabetes and its associated risk factors, which are increasingly prevalent in older adults. This study also has limitations. First, more than 80% of participants had type 2 diabetes; so results may not be generalized to the general population. Second, the initial (baseline) participants were older

and less highly educated, and had lower BMI, more hypertension, and less type 2 diabetes. As such, their study results should be compared to the full DHS-Mind sample with caution. Third, no causal relationship regarding to the mediation effect of vascular calcification can be established due to the cross-sectional design. Fourth, we attempted to perform formal mediation tests using the method developed by Bauer et al. (51). However, all models did not converge, except when Phonemic Fluency was treated as the dependent variable. The mediation effects (i.e., indirect effects) were not significant on Phonemic Fluency (all  $p$ -values  $> 0.05$ ); therefore we did not present these test results in the manuscript.

## CONCLUSION

Overall, this study demonstrated multiple relationships between neuroimaging measures and cognitive testing in a type 2 diabetes-enriched population. These associations were slightly attenuated, but not eliminated, after adjusting for subclinical CVD, measured as CAC. Additional research is necessary to understand how atherosclerosis interacts with other factors, such as genetic factors and glycemic control, to injure the brain and impair cognitive function.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

### FUNDING

This study was supported in part by the National Institutes of Health through R01 HL67348, R01 HL092301, R01 NS058700 (to DWB), R01 NS075107 (to BIF, JAM), F32 DK083214-01 (to CEH), F31 AG044879 (to LMR) and the General Clinical Research Centre of the Wake Forest School of Medicine (M01 RR07122, F32 HL085989). The authors thank the other investigators, the staff, and the participants of the DHS study for their valuable contributions.

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Demographic characteristics of all European American participants recruited to the DHS-Mind study (n=893) and the subgroup previously enrolled in initial examination (n=572)

Table 1

	DHS-Mind Sample (n=893) <sup>3</sup>		Initial Enrollment (n=572) <sup>3</sup>		p-value <sup>2</sup>
	Mean±SD or %	Median (range)	Mean±SD or %	Median (range)	
<b>Demographic Information</b>					
Age (years)	65.8±9.7	66.3(37.7–93.2)	67.3±8.9	67.5(41.3–89.2)	1.5×10 <sup>-9</sup>
Gender (% female)	52.9%		54.6%		0.054
BMI (kg/m <sup>2</sup> )	32.5±6.7	31.4(14.6–59.0)	31.6±6.6	30.4(17.6–58.4)	6.3×10 <sup>-6</sup>
% smoking (current or past)	54.6%		55.9%		0.746
Hypertension (%)	86.9%		88.1%		0.027
Systolic blood pressure(mmHg)	130.3±17.8	128(86–216)	132±18.1	131(89–216)	1.3×10 <sup>-6</sup>
Diastolic blood pressure (mmHg)	71.4±10.4	71(39–121)	70.2±10.4	70(39–121)	1.4×10 <sup>-6</sup>
Self-reported history of prior CVD	34.9%		33.5%		0.991
CES-D score	7.5±5.3	6 (0–29)	7.1±5.2	6 (0–29)	0.021
<b>Type 2 Diabetes</b>					
Type 2 diabetes affected (%)	87.6%		80.9%		4.6×10 <sup>-18</sup>
Diabetes Duration (years)	15.4±7.7	13.6(0.4–45.0)	16.6±7.0	14.7(1.9–44.5)	7.4×10 <sup>-7</sup>
Glucose (mg/dL) (%)	54.4	127(40–408)		121(40–349)	5.2×10 <sup>-5</sup>
Hemoglobin A1c	141.2±7.3±1.4	7(1.9–14.8)	134±48.9 7.1±1.3	6.8(4.9–14.8)	2.5×10 <sup>-4</sup>
<b>Medication Use</b>					
Anti-diabetic Medication <sup>1</sup>	73.2%		67.1%		2.1×10 <sup>-6</sup>
Cholesterol-lowering Medication	52.4%		53.7%		0.184
Anti-hypertensive Medication	67.4%		73.1%		1.3×10 <sup>-5</sup>
<b>Education</b>					
Less than high school	14.8%		17.8%		4.9×10 <sup>-10</sup>
High school	47.7%		53.9%		
Greater than high school	37.5%		28.3%		



	DHS-Mind Sample (n=893) <sup>3</sup>		Initial Enrollment (n=572) <sup>3</sup>		p-value <sup>2</sup>
	Mean±SD or %	Median (range)	Mean±SD or %	Median (range)	
<b>Lipids</b>					
Total cholesterol (mg/dL)	185.5±41.8	181(65–391)	185.5±41.8	181(65–391)	
HDL cholesterol (mg/dL)	43.4±11.6	42(14–102)	43.4±11.6	42(14–102)	
LDL cholesterol (mg/dL)	104.9±32.1	104(14–221)	104.9±32.1	104(14–221)	
Triglycerides	192.9±128.8	161(30–1310)	192.9±128.8	161(30–1310)	
<b>Coronary Artery Calcified Plaque (CAC)</b>					
	1290.2±3189.4	204(0–50415)	1290.2±3189.4	204(0–50415)	
<b>Cognitive Function Test Scores</b>					
Modified Mini Mental State Exam (3MSE)	90.9±7.1	92(43–100)	90.4±7.3	92(43–100)	0.001
Digit Symbol Substitution (DSST)	50±16	50(10–106)	47.6±15.3	47(10–98)	9.7×10 <sup>-9</sup>
Stroop	34.2±19.6	29(2–161)	35.8±20.3	30(2–161)	5.2×10 <sup>-5</sup>
Rey Auditory-Verbal Learning Task (RAVLT)	41.2±10.2	41(11–66)	41.8±10.2	42(11–66)	0.241
Phonemic Fluency	29.7±11.7	29(2–67)	28.7±11.6	28(2–67)	8.2×10 <sup>-4</sup>
Semantic Fluency	30.8±8.5	30(11–69)	29.8±7.9	29(11–60)	9.9×10 <sup>-6</sup>
<b>Imaging</b>					
Total brain volume (TBV) (cc)	1092±112	1080(775–1448)	1085±113	1072(775–1448)	0.009
White matter lesion volume (WMLV) (cc)	4.5±8.3	1.5(0–103.3)	4.4±7.1	1.6(0–59.6)	0.060
Gray matter volume (GMV) (cc)	519±54	517(373–709)	511±51	508(375–660)	1.4×10 <sup>-8</sup>
White matter volume (WMV) (cc)	573±70	568(400–815)	574±71	569(400–815)	0.603
Total intracranial volume (ICV) (cc)	1350±138	1337(985–1759)	1340±139	1328(985–1759)	0.002
Fractional anisotropy white matter (WMFA)	0.35±0.03	0.35(0.26–0.42)	0.36±0.02	0.36(0.29–0.42)	9.4×10 <sup>-20</sup>
Fractional anisotropy gray matter (GMFA)	0.20±0.02	0.20(0.14–0.35)	0.21±0.02	0.21(0.15–0.26)	9.5×10 <sup>-18</sup>
Mean diffusivity white matter (WMMD)	0.79±0.05	0.79(0.63–0.95)	0.79±0.05	0.79(0.63–0.95)	0.162
Mean diffusivity gray matter (GMMD)	1.09±0.09	1.08(0.68–1.38)	1.09±0.09	1.09(0.80–1.38)	0.104
Gray matter cerebral blood flow (GMCBF) (mL/100g of tissue/min)	45.0±17.3	42.8(9.0–134.9)	46.7±18.2	44.1(9.1–134.9)	5.9×10 <sup>-5</sup>

<sup>1</sup> either oral hypoglycemic medications or insulin

<sup>2</sup> The comparison between those who were in the initial enrollment and those who were not using marginal models

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<sup>3</sup> Some variables have missing data, so the sample size for each variable is listed below. The sample size for DHS-Mind sample: BMI 891; CES-D score 749; type 2 diabetes duration 745; glucose and HbA1c 845; 3MSE 892; DSST 889; Stroop 883; RAVLT 891; fluency: 849; TBV 717; WMLV 742; GMV, WMV, and ICV 718; WMFA, GMFA, WMMD, and GMMD 671; GMCBF 615. The sample size for the initial enrollment cohort: BMI 571; CES-D score 429; type 2 diabetes duration 429; glucose and HbA1c 527; coronary calcified plaque 532; 3MSE 571; DSST 569; Stroop 565; RAVLT 570; semantic and phonemic fluency: 529; WMLV 448; GMV, WMV, TBV, and ICV 480; WMFA, GMFA, WMMD, and GMMD 476; GMCBF 449.

Table 2

Association between cognitive performance and brain imaging for the subset with a baseline exam<sup>1</sup>

Variable	3MSE <sup>2</sup>	DSST <sup>2</sup>	Stroop <sup>3</sup>	RAVLT <sup>2</sup>	Phonemic Fluency	Semantic Fluency
TBV <sup>23</sup>	β Estimate (CI) 4.43 (0.17, 8.69)	9.89 (4.62, 15.17)	-0.17 (-0.49, 0.15)	3.86 (0.72, 6.99)	5.84 (1.29, 10.31)	5.00 (2.11, 7.90)
	p-value 0.042	<b>2.4 x 10<sup>-4</sup></b>	0.296	0.016	0.012	<b>7.0 x 10<sup>-4</sup></b>
WMLV <sup>23</sup>	β Estimate (CI) -0.23 (-0.93, 0.47)	-2.55 (-3.83, -1.26)	0.02 (-0.04, 0.07)	-0.95 (-1.88, -0.02)	-1.07 (-2.30, 0.16)	-1.20 (-2.02, -0.39)
	p-value 0.518	<b>1.0 x 10<sup>-4</sup></b>	0.511	0.046	0.087	0.004
GMV <sup>2</sup>	β Estimate (CI) 2.86 (1.21, 4.52)	3.01 (0.64, 5.39)	-0.05 (-0.18, 0.08)	2.13 (0.43, 3.82)	2.77 (0.62, 4.91)	2.46 (1.01, 3.91)
	p-value <b>7.0 x 10<sup>-4</sup></b>	0.013	0.463	0.014	0.011	<b>9.0 x 10<sup>-4</sup></b>
WMV <sup>23</sup>	β Estimate (CI) -0.28 (-2.11, 1.55)	2.66 (-0.65, 5.97)	-0.06 (-0.19, 0.06)	-0.09 (-2.24, 2.06)	0.72 (-2.28, 3.72)	0.44 (-1.45, 2.32)
	p-value 0.767	0.115	0.314	0.935	0.639	0.651
WMFA <sup>2</sup>	β Estimate (CI) 0.77 (0.07, 1.47)	3.26 (1.83, 4.70)	-0.09 (-0.15, -0.04)	0.99 (0.05, 1.94)	1.54 (0.10, 2.98)	1.42 (0.54, 2.30)
	p-value 0.031	<b>8.0 x 10<sup>-6</sup></b>	<b>4.6 x 10<sup>-4</sup></b>	0.040	0.036	1.6 x 10 <sup>-3</sup>
GMCBF <sup>23</sup>	β Estimate (CI) -0.70 (-1.30, -0.11)	0.29 (-0.89, 1.46)	-0.04 (-0.08, 0.01)	0.62 (-0.20, 1.44)	0.81 (-0.27, 1.88)	0.41 (-0.44, 1.25)
	p-value 0.021	0.633	0.131	0.138	0.140	0.348

<sup>1</sup> All models adjusted for age, gender, educational attainment, type 2 diabetes, and hypertension; TBV, WMLV, GMV, and WMV additionally adjusted for intracranial volume

<sup>2</sup> 3MSE: Modified Mini Mental State Exam; DSST: Digit Symbol Substitution; RAVLT: Rey Auditory-Verbal Learning Task; TBV: Total brain volume; WMLV: Total white matter lesion volume; GMV: Gray matter volume; WMV: White matter volume; WMFA: Fractional anisotropy white matter; GMCBF: Gray matter cerebral blood flow

<sup>3</sup> Stroop, TBV, WMLV, and WMV are log transformed. GMCBF is squared root transformed.

**Table 3**

Association between standardized coronary artery calcified plaque and cognitive function and brain imaging (standardized) for the subset with a baseline exam<sup>1</sup>

Variable	$\beta$ Estimate (95% Confidence Interval)	p-value
<b>Cognitive Function Test Measures</b>		
3MSE <sup>2</sup>	0.09 (-0.53, 0.71)	0.776
DSST <sup>2</sup>	-1.38 (-2.58, -0.18)	0.024
Stroop <sup>3</sup>	0.04 (-0.01, 0.08)	0.104
RAVLT <sup>2</sup>	-1.27 (-2.12, -0.42)	<b>0.003</b>
Phonemic Fluency	0.17 (-0.90, 1.24)	0.751
Semantic Fluency	-0.74 (-1.43, -0.06)	0.034
<b>Imaging Measures</b>		
TBV <sup>23</sup>	-0.05 (-0.07, -0.03)	<b>6.0 x 10<sup>-6</sup></b>
WMLV <sup>23</sup>	0.12 (0.02, 0.21)	0.023
GMV <sup>2</sup>	-0.08 (-0.13, -0.04)	<b>0.0007</b>
WMV <sup>23</sup>	-0.01 (-0.05, 0.03)	0.493
WMFA <sup>2</sup>	-0.02 (-0.10, 0.07)	0.679
GMCBF <sup>23</sup>	0.04 (-0.08, 0.15)	0.512

<sup>1</sup> All cognitive function models adjusted for age, gender, educational attainment, type 2 diabetes, and hypertension; All imaging models adjusted for the same covariates except educational attainment; TBV, WMLV, GMV, and WMV additionally adjusted for intracranial volume

<sup>2</sup> 3MSE: Modified Mini Mental State Exam; DSST: Digit Symbol Substitution; RAVLT: Rey Auditory-Verbal Learning Task; TBV: Total brain volume; WMLV: Total white matter lesion volume; GMV: Gray matter volume; WMV: White matter volume; WMFA: Fractional anisotropy white matter; GMCBF: Gray matter cerebral blood flow

<sup>3</sup> Coronary calcified plaque, Stroop, TBV, WMLV, and WMV are log transformed. GMCBF is squared root transformed.

Association of cognitive performance and brain imaging (standardized) after adjusting for standardized coronary calcified plaque and other covariates for the subset with a baseline exam<sup>1</sup>

Table 4

Imaging measure	3MSE <sup>2</sup>	DSST <sup>2</sup>	Stroop <sup>3</sup>	RAVLT <sup>2</sup>	Phonemic Fluency	Semantic Fluency
TBV <sup>23</sup>	$\beta$ Estimate (CI) 4.77 (0.55, 8.99)	10.01 (4.42, 15.59)	-0.16 (-0.50, 0.19)	3.58 (0.34, 6.82)	6.23 (1.56, 10.91)	5.07 (1.95, 8.18)
	p-value 0.027	<b><math>4.5 \times 10^{-4}</math></b>	0.375	0.030	0.009	<b>0.001</b>
WMLV <sup>23</sup>	$\beta$ Estimate (CI) -0.36 (-1.08, 0.35)	-2.71 (-4.01, -1.40)	0.01 (-0.04, 0.07)	-0.91 (-1.86, 0.05)	-1.07 (-2.33, 0.19)	-1.29 (-2.11, -0.48)
	p-value 0.321	<b><math>5.1 \times 10^{-5}</math></b>	0.679	0.063	0.096	0.002
GMV <sup>2</sup>	$\beta$ Estimate (CI) 2.98 (1.22, 4.74)	2.72 (0.25, 5.18)	-0.03 (-0.16, 0.10)	1.84 (0.14, 3.54)	2.70 (0.48, 4.91)	2.16 (0.69, 3.62)
	p-value <b><math>9.3 \times 10^{-4}</math></b>	0.031	0.633	0.034	0.017	0.004
WMV <sup>23</sup>	$\beta$ Estimate (CI) -0.26 (-1.94, 1.42)	2.81 (-0.54, 6.16)	-0.07 (-0.20, 0.06)	0.04 (-2.12, 2.20)	0.85 (-2.29, 3.99)	0.71 (-1.18, 2.61)
	p-value 0.763	0.100	0.317	0.968	0.597	0.461
WMFA <sup>2</sup>	$\beta$ Estimate (CI) 0.79 (0.10, 1.49)	3.20 (1.76, 4.63)	-0.09 (-0.14, -0.04)	1.09 (0.13, 2.04)	1.44 (-0.01, 2.89)	1.48 (0.61, 2.35)
	p-value 0.026	<b><math>1.3 \times 10^{-5}</math></b>	<b><math>9.8 \times 10^{-4}</math></b>	0.026	0.052	<b><math>8.7 \times 10^{-4}</math></b>
GMCBF <sup>23</sup>	$\beta$ Estimate (CI) -0.66 (-1.30, -0.02)	0.40 (-0.69, 1.50)	-0.04 (-0.09, 0.01)	0.75 (-0.05, 1.55)	0.73 (-0.36, 1.81)	0.47 (-0.39, 1.33)
	p-value 0.043	0.469	0.121	0.065	0.189	0.282

<sup>1</sup> All models adjusted for age, gender, educational attainment, type 2 diabetes, hypertension, and coronary calcified plaque; TBV, WMLV, GMV, and WMV additionally adjusted for intracranial volume

<sup>2</sup> 3MSE: Modified Mini Mental State Exam; DSST: Digit Symbol Substitution; RAVLT: Rey Auditory-Verbal Learning Task; TBV: Total brain volume; WMLV: Total white matter lesion volume; GMV: Gray matter volume; WMV: White matter volume; WMFA: Fractional anisotropy white matter; GMCBF: Gray matter cerebral blood flow

<sup>3</sup> Stroop, coronary calcified plaque, TBV, WMLV, and WMV are log transformed. GMCBF is squared root transformed.