Baseline Cardiovascular Risk Predicts Subsequent Changes in Resting Brain Function

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Background and Purpose—The Framingham Heart Study group cardiovascular disease risk profile (FCRP) score was used to assess the relationship between baseline cardiovascular risk and subsequent changes in resting state cerebral blood flow (CBF) in cognitively normal older participants from the Baltimore Longitudinal Study of Aging.

Methods—Ninty-seven cognitively normal participants underwent annual resting-state positron emission tomography scans at baseline and over a period of up to 8 years (mean interval, 7.4 years). Images quantifying voxel-wise longitudinal rates of CBF change were calculated and used to examine the relationship between baseline FCRP score and changes over time in regional CBF. Individual components of the FCRP score (age, cholesterol, blood pressure, smoking status, and type 2 diabetes) were also correlated with changes in regional CBF to examine the independent contributions of each component to the overall pattern of change.

Results—Higher baseline FCRP scores were associated with accelerated longitudinal decline in CBF in orbitofrontal, medial frontal/anterior cingulate, insular, precuneus, and brain stem regions. Of the components that comprise the FCRP score, higher diastolic blood pressure and diabetes were associated independently with greater decline in the medial frontal/anterior cingulate and insular regions, respectively.

Conclusions—Baseline cardiovascular risk factors are associated with greater rates of decline in resting state regional brain function. The regions showing accelerated decline participate in higher-order cognitive processes and are also vulnerable to age-related neuropathology. These results, in conjunction with other studies, encourage early treatment of cardiovascular risk factors in older individuals. (Stroke. 2012;43:00-00.)

Key Words: aging ■ cholesterol ■ diabetes ■ hypertension ■ positron emission tomography

A large body of evidence supports a relationship between cardiovascular risk factors and cognitive impairment in the elderly. These factors include atherosclerosis, hypertension, coronary artery disease, dyslipidemia, and diabetes mellitus. Several studies also suggest that vascular factors associated with increased cardiovascular risk lead to earlier onset and faster progression of late-onset dementias. Although the precise temporal relationship between the development of cardiovascular risk factors and subsequent dementia remains unclear, previous reports indicate that elevated serum cholesterol levels and hypertension in midlife are associated with increased risk of Alzheimer disease (AD). These findings suggest that vascular risk factors modulate early events in AD pathogenesis that precede the onset of overt cognitive impairment by many years. Associations between midlife vascular risk and later cognitive impairment are of considerable public health importance because they indicate that primary preventive strategies targeting reduction in cardiovascular risk may, in turn, also delay the onset or decrease the burden of late-onset dementia. Further emphasizing the importance of vascular risk factors in aging, the American Heart Association/American Stroke Association recently issued a statement to healthcare professionals suggesting that long-term vascular risk marker interventional studies may be required to prevent or postpone the onset of vascular cognitive impairment and AD. Thus, as the field of age-related neuroimaging has begun to focus on early predictors of future change, there is considerable interest in identifying early changes in brain structure and function associated with future cognitive outcome in older individuals.

Using serial $^15$O-water positron emission tomography (PET) data from the neuroimaging substudy of the Baltimore Longitudinal Study of Aging, we previously reported that hypertension was associated with significant longitudinal decreases in regional cerebral blood flow (rCBF) in a select group of older individuals with no other comorbidities. The aim of the present study was to further investigate the relationship between risk for cardiovascular disease and...
Table 1. Participant Demographics

<table>
<thead>
<tr>
<th>N</th>
<th>Baseline age, y</th>
<th>69.5±7.2 (57–86)</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>58</td>
</tr>
<tr>
<td>Annual scans</td>
<td>8.2±1.0 (5–9)</td>
<td></td>
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<tr>
<td>Scan on interval</td>
<td>7.4±1.0 (4–8)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>138.8±20.8 (100–192)</td>
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<tr>
<td>Diastolic BP</td>
<td>81.7±10.4 (55–110)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>213.6±33.3 (149–343)</td>
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</tr>
<tr>
<td>LDL</td>
<td>115.2±32.2 (46–249)</td>
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</tr>
<tr>
<td>HDL</td>
<td>50.4±15.2 (22–95)</td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td>15</td>
<td></td>
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<tr>
<td>Diabetes</td>
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<td></td>
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<tr>
<td>Education, y</td>
<td>16.2±2.8 (8–20)</td>
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<tr>
<td>MMSE baseline</td>
<td>28.8±1.3 (23–30)</td>
<td></td>
</tr>
<tr>
<td>MMSE last visit</td>
<td>28.9±1.2 (24–30)</td>
<td></td>
</tr>
</tbody>
</table>

Mean values are listed for all participants. The range of scores is shown in parentheses. MMSE scores are also shown.

BP indicates blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; MMSE, Mini Mental State Examination.

longitudinal changes in rCBF, an indirect marker of neuronal activity in the human brain.15

In this study, we assess the relationship between a composite measure of cardiovascular risk (the Framingham cardiovascular disease risk profile [FCRP] score) calculated at baseline and subsequent longitudinal changes in rCBF over a 7-year period in a sample of 97 cognitively normal older adults. We also investigated the contributions of individual risk factors to these longitudinal patterns of rCBF change. Based on previously observed associations between vascular risk factors and dementia, we hypothesized that baseline cardiovascular risk would be associated with changes in rCBF over time in brain regions vulnerable to AD pathology, even in normal older individuals.

Subjects and Methods
We used PET data from 97 cognitively normal older participants (58 males; mean age at baseline, 69.5; SD, 7.2) in the neuroimaging substudy16 of the Baltimore Longitudinal Study of Aging17 (Table 1). These individuals are considered typical agers, with no history of central nervous system disease (epilepsy, stroke), psychiatric disorders, severe cardiac disease (myocardial infarction, coronary artery disease requiring angioplasty or bypass surgery), or metastatic cancer. Individuals with clinical diagnoses of mild cognitive impairment or dementia at the beginning of the study or who had development of either mild cognitive impairment or dementia during the study were also excluded from the analyses.

This study was approved by the local Institutional Review Board. All subjects provided written informed consent before each assessment.

Measures of Cardiovascular Risk
At regular Baltimore Longitudinal Study of Aging visits, medical examinations were performed to determine health status. Medical histories were recorded that included assessment of age, presence of type 2 diabetes, and smoking status. Measures of cholesterol (total and high-density lipoprotein), and systolic and diastolic blood pressure were also collected. These measures were used to calculate the FCRP score at the baseline imaging visit.

Neuropsychological Testing
During each neuroimaging visit, participants completed the Mini-Mental State Examination and a battery of 12 neuropsychological tests evaluating 6 cognitive domains. Memory was assessed using the California Verbal Learning Test and Benton Visual Retention Test. Word knowledge and verbal ability were measured using Primary Mental Abilities Vocabulary. Verbal fluency was assessed by letter (ie, FAS) and category fluency tests. Attention and working memory were measured by the Digit Span Test of the Wechsler Adult Intelligence Scale Revised and the Trail Making Test. Digits Backward, Trails B, and Verbal Fluency (categories and letters) assessed executive function. The Card Rotations Test assessed visuospatial function.

Data from evaluations at baseline through the last annual PET visit were used to examine the relationship between baseline FCRP score and cognitive performance. Linear mixed effects regression models were used to investigate the effects of baseline FCRP scores on baseline cognitive performance and on longitudinal rates of change in cognitive performance. We used cognitive performance measures as dependent variables and FCRP risk score, baseline age, sex, time interval (follow-up time from baseline), and the interactions between these variables as predictors.

PET Scanning
Participants underwent PET scans at baseline (year 1) and up to 8 annual follow-up scans. Each session included a resting PET scan in which participants were instructed to keep their eyes open and focused on a computer screen covered by a black cloth.

PET measures of rCBF were obtained using $^{15}$O water. For each scan, 75 mCi of $^{15}$O water were injected as a bolus. Scans were performed on a GE 4096 scanner, which provides 15 slices of 6.5-mm thickness. Images were acquired for 60 seconds from the time the total radioactivity counts in the brain reached threshold level. Attenuation correction was performed using a transmission scan acquired before the emission scan.

PET Data Analysis
Data from PET scans obtained annually from baseline to the last available follow-up time points were used in the analyses. The mean interval between baseline and last follow-up PET scan was 7.4 (SD ±1.0) years. The PET scans were realigned and spatially normalized into standard stereotactic space and smoothed to full width at half maximum of 12x12x12 mm in the x, y, and z planes. Next, to control for variability in global flow, rCBF values at each voxel were ratio-adjusted to the mean global flow and scaled to 50 mL/100 g/min for each scan, and then thresholded to exclude peripheral signal scatter in the images. For each participant, rates of change in cerebral blood flow (CBF) were calculated across all preprocessed scans using voxel-wise linear modeling and extraction of the estimated fit parameter. An image of the voxel-wise longitudinal rates of change (ie, slope or linear temporal trends image) was then created for each subject (Statistical Parametric Mapping software, SPM2; Wellcome Trust Centre for Neuroimaging, University College London).

The slope images from all subjects were used in a voxel-based multiple regression analysis (SPM5) with baseline FCRP score as an independent predictor of longitudinal change in rCBF. The associations were adjusted for baseline age, sex, and the interval between baseline and last scan. In secondary analyses, we examined the contributions of individual components of the FCRP score to the predicted pattern of change from the overall analysis. In a restricted search of regions from the first analysis, age, cholesterol, systolic and diastolic blood pressure, diabetes, and smoking status were regressed with CBF changes individually while controlling for the other factors to examine the independent contributions of each component to the overall pattern of change. Significant associations were based on a statistical threshold of $P<0.005$ and restricted to regions with a spatial extent of at least 50 voxels.

To determine the annual rates of change in CBF for each region, rCBF values were extracted from a 4-mm spherical region centered on the local...
maxima of significant areas using the Marsbar SPM toolbox. To determine if change in tissue volume in each specific region had an effect on the decline in CBF of that region, we repeated all analyses while controlling (covarying) for the annual rate of change in MRI volume of each cluster observed if change in tissue volume in each specific region had an effect on the declines in CBF of that region, we repeated all analyses while controlling (covarying) for the annual rate of change in MRI volume of each cluster observed if change in tissue volume in each specific region had an effect on the

MRI Scanning
Scanning was performed on a GE Signa 1.5-Tesla scanner. A 3-dimensional T1-weighted spoiled gradient refocused MRI scan (35 ms repetition time; 5 ms echo time; 24 cm field of view; 45° flip angle; 256×256 matrix; 0.94×0.94 mm voxel size; 1.5-mm slice thickness; 124 slices) was obtained annually at each imaging visit.

MRI Volume Calculation
The MRI scans were segmented into gray matter, white matter, and cerebrospinal fluid and spatially normalized into stereotactic space using a high-dimensional elastic warping method and a volume-preserving transformation. Binary maps of the clusters showing an association between cardiovascular risk and declines in CBF were generated from the PET analysis, and total volumes of gray plus white matter were calculated within each cluster for each participant. The annual rate of volume change within each region was estimated using linear mixed models. The rate of change for each cluster was then included as a covariate in the follow-up PET analysis.

Results
Cardiovascular Risk Scores
FCRP scores were calculated at year 1 baseline, yielding a mean FCRP baseline score of 13.2 (SD, 7.5) with a range of 3 to 27 using the formula described by Wilson et al. FCRP Variables and rCBF Change
We performed secondary analysis of the individual components of the FCRP score (age, smoking status, cholesterol, blood pressure, and type 2 diabetes) to identify the specific factors accounting for the regional associations between FCRP and change in CBF. Within the regions showing significant changes in the primary analysis, several components of the FCRP showed significant independent associations with rCBF change. Higher baseline diastolic blood pressure was associated with greater decline in rCBF within the medial frontal/anterior cingulate region, and diabetes was associated with greater decrease in insular CBF (Figure 3). These results did not change when controlling for longitudinal tissue volume decline within these regions.

Discussion
Using the FCRP, which includes measures of age, cholesterol levels, blood pressure, diabetes, and smoking status, we found that higher baseline FCRP scores were associated with greater longitudinal CBF decline in orbitofrontal, medial frontal/anterior cingulate, insular, precuneus, and brainstem regions. Although we see little effect of FPRC score on cognition in this sample of cognitively normal individuals, these regions are known to participate in higher-order cognitive processes and are also regions of pathological change in AD. Whereas CBF decline in these regions was associated with the overall cardiovascular risk score, decreased CBF in medial frontal/anterior cingulate and insular regions was additionally related to independent components of the risk score.
Although previous findings suggest that the FCRP is not associated with measures of global CBF, our observation of association between higher cardiovascular risk and decline in frontal cerebral blood flow is consistent with a study examining the relationship between FCRP and brain glucose metabolism. In the latter investigation, increased risk scores were associated with decreased glucose metabolism in medial frontal, orbitofrontal, and inferior frontal/insular areas. Together, these findings suggest that frontal lobe regions are susceptible to functional decline over time in older individuals with a higher risk of development of cardiovascular disease. The brain regions showing associations between FCRP and rCBF include areas involved in attention, error detection, and performance monitoring processes and suggest aspects of cognition that may show future vulnerability to cardiovascular risk. Although there was no significant effect of risk score on rates of decline in cognitive...
performance over the study interval, changes in brain function may occur before changes in cognitive function become apparent.

Declines in rCBF were also observed in the precuneus. Decreased glucose metabolism in the posterior cingulate/precuneus is one of the earliest correlates of abnormal neuronal function in AD.26,27 This region also exhibits functional MRI signal changes in individuals with mild cognitive impairment before the onset of AD,28 as well as neurofibrillary tangle and diffuse amyloid accumulation in AD demonstrated both pathologically29,30 and with in vivo fibrillar amyloid tracers.31

Interestingly, we have previously shown that amyloid burden significantly increases over time in nondemented older individuals. This increase was seen not only in the precuneus region but also in the orbitofrontal cortex and anterior cingulate/medial frontal areas32 that show significant decrements in rCBF in relation to cardiovascular risk in the present study. This is particularly important because recent evidence points to a link between vascular dysfunction and the development of AD pathology,33 and especially amyloid accumulation.6 Because the associations between decreased CBF and increased risk in frontal and precuneus regions were observed in currently cognitively normal individuals, these findings may be an early marker of neurodegenerative changes that occur not only as a result of AD but also in conjunction with vascular dysfunction, and could ultimately result in cognitive decline.34 Taken together, these findings indicate that cardiovascular risk factors may influence neuronal function within regions susceptible to pathological change in the aging brain.

Of the components that comprise the FCRP score, higher blood pressure was associated with greater CBF decline in medial frontal/anterior cingulate cortex. This was true for diastolic pressure and is in agreement with previous results from our laboratory that showed greater CBF decline over a 6-year period in this region in individuals with hypertension relative to those with normal blood pressure.14 It is also consistent with a study by Dai et al35 in which older individuals with hypertension exhibited decreased CBF in the anterior cingulate. Type 2 diabetes was independently associated with greater insular CBF decrease over time. Few neuroimaging studies have examined the effects of diabetes on brain function, but some studies have shown a relationship between diabetes and white matter changes in the brain36,37 that could contribute to the functional decline observed in the insula.

Several studies have also noted structural changes related to vascular risk scores. Increased risk scores have been associated with decreased whole brain volumes and regional volume of the frontal lobes.38 Hypertension alone has also been associated with decreased medial frontal and anterior cingulate volumes,39,40 whereas diabetes has been linked with decreased frontal lobe volumes, including inferior regions that comprise the medial wall of the insular cortex.41,42 Although the current findings suggest that volumetric changes in localized clusters of functional change do not play a significant role in the accelerated CBF decline in those regions, decreased volume in frontal lobe structures has been associated with increased vascular risk in previous studies.

Together, these results show that baseline cardiovascular risk factors are associated with future declines in regional brain function over time. In this sample, however, baseline FCRP scores were not related to rates of decline in cognitive performance over time. This may reflect the fact that these participants represent a relatively healthy subset who maintained cognitive health over time, because participants with even mild cognitive impairment were excluded from these analyses. In addition, participants with severe risk factors for cardiovascular disease were excluded from the neuroimaging study at its inception, further restricting the range of FCRP and cognitive performance. It is also important to note that our study collected only 1 resting scan per year. Trajectories of CBF change, however, were based on as many as 9 longitudinal scans, increasing the stability of our longitudinal estimates. Nevertheless, variance associated with acquisition of a single scan per year could reduce our power to detect additional associations.

Future investigations over longer follow-up intervals that include individuals who have development of cognitive impairment will help determine whether these CBF changes will ultimately lead to cognitive decline in those at higher risk for development of cardiovascular disease. Such studies are important because emerging findings suggest that the treatment of factors such as hypertension and diabetes may reduce the risk of subsequent cognitive and functional decline43,44 in older individuals.

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