Use of Total Cerebral Blood Flow as an Imaging Biomarker of Known Cardiovascular Risks

J. Richard Jennings, PhD; Alicia F. Heim, BS; Dora Chieh-Hsin Kuan, MS; Peter J. Gianaros, PhD; Matthew F. Muldoon, MD, MPH; Stephen B. Manuck, PhD

Background and Purpose—This study examined whether overall cerebral blood flow was associated with known vascular risk factors, including cardiometabolic risk factors that comprise the metabolic syndrome, carotid artery intima-media thickness, and the Framingham risk score.

Methods—Three separate samples were available for analysis. Two comparable samples were combined to form a primary sample of middle-aged participants (n=576; 30–55 years of age) that completed both a risk factor assessment and a resting brain scan. Samples were recruited via mailings and advertisements within an urban area. Quantitative measures of cerebral blood flow were derived from arterial spin–labeled MRI in this sample and in a validation/generalization sample (n=76; 30–55 years).

Results—Cerebral blood flow was inversely associated with cardiometabolic risk indices, that is, associated with lower waist circumference, systolic blood pressure, glucose, and triglyceride and high-density lipoprotein. Moreover, cerebral blood flow was also related to Framingham risk and carotid intima-media thickness. In the validation sample, which used a slightly different brain imaging technique, significant relationships were replicated for cardiometabolic risk, but not for Framingham risk.

Conclusions—Reduced cerebral blood flow seems to be a correlate of vascular disease risk factors associated with cardiometabolic dysregulation. Cerebral blood flow may provide a valid imaging biomarker for cardiovascular risk. (Stroke. 2013;44:2480-2485.)

Key Words: biological markers • brain diseases, metabolic • carotid intima-media thickness • cerebral blood flow • Framingham risk • magnetic resonance imaging • neuroimaging • vascular diseases

Resting cerebral blood flow (CBF) shows substantial stability attributable to autoregulation, but decrements in CBF with age and vascular disease may underlie cognitive impairments and dementia. This raises the question of whether vascular risk factors relate to cerebrovascular circulation before old age.

This question remains largely open because total CBF is not routinely assessed because of the nonquantitative nature of typical MRI measures, the expense of positron emission tomography assessment, and a research focus on elderly samples. Early work with small samples using xenon inhalation and single photon tomography provided estimates of CBF and suggested a decline in CBF with age, with trends toward exacerbation of this decline by cardiovascular risk factors. One larger, longitudinal study verified the CBF decline with age as well as demonstrating a CBF decline with overt cerebrovascular disease. A small number of more current studies variously estimating CBF also suggest a relationship to vascular risk factors. Among elderly men, but not women, blood pressure (BP) is negatively related to single photon emission–computed tomography regional CBF estimates. Hypertension among patients with atherosclerosis has been shown during 5 years of follow-up to reduce CBF estimated from arterial measures. Effective treatment of hypertension has been shown, however, to enhance CBF velocity. Less CBF velocity has also been related to the presence of type 2 diabetes mellitus and inflammatory indices. These arterial velocity measures also show lower estimated CBF to relate to greater number of white matter hyperintensities. Framingham risk has been related to longitudinal reductions in regional flow to visceromotor and viscerosensory brain areas using relative measures from positron emission tomography, to middle cerebral artery flow, and to cerebral vasoreactivity to hypercapnia, but not to overall CBF, as assessed with arterial spin labeling (ASL). In short, quantitative resting CBF has rarely been examined relative to cardiovascular risk, although various less direct techniques and related measures suggest that a relationship will be observed.

Moreover, virtually all evidence to date bearing on this issue has been from elderly participants (mean ages, ≥65 years). Vascular risk factors emerge over time and are useful to identify...
before frank cerebrovascular disease. The current study cross-sectionally examined in midlife samples (30–55 years of age) whether components of the metabolic syndrome (MetS)\(^9\) would relate to total CBF. Additional analyses examined the relationship to carotid intima thickness\(^\text{20,21}\) and the Framingham risk score.\(^7\) The use of ASL, an MRI technique yielding quantitative CBF estimates, permitted examination of a reasonably large sample.\(^3\)–\(^6\) Total CBF was expected to relate inversely to risk. Emphasis was placed on a quantitative combination of MetS components, given the expected low prevalence of the complete MetS in the midlife. We tested our hypothesis in the primary sample and examined the robustness of our results in an independent, more racially diverse sample imaged by pseudo-continuous ASL rather than pulsed ASL MRI sequence. This sample also had measures of white matter hyperintensity available. We sought in the validation sample to replicate relationships between CBF and MetS components despite any variance attributable to the differing ASL sequences.

### Methods

#### Participants

We report on 576 participants with acceptable brain imaging and risk data drawn from 2 projects. Table 1 presents participant characteristics separately for the 2 samples combined and third, validation sample. Inclusion criteria are listed in the online-only Data Supplement; those with cardiovascular disease or medicated for this, diabetes mellitus, or lipids were excluded. The validation sample included participants collected to date from a study of middle-aged participants with normal to prehypertensive levels of BP. Seventy-six participants were available. Other than the BP inclusion, inclusion criteria were essentially identical to the other studies. Cardiometabolic risk (CMR) measures were assessed using the same methods as in the primary sample. All studies used an ASL technique, but the studies in the primary sample used the pulsed ASL MRI sequence and validation sample used pseudocontinuous ASL. Psychosocial and psychophysiological predictors of cardiovascular risk were the focus of the studies in the primary sample and progression to hypertension, the focus of the validation study. Further minor differences in age and racial composition were adventitious. Samples were recruited via mailings and advertisements within an urban area, mass mailings to targeted areas, and campus and city newspaper advertisements. Participants in all studies provided informed consent, and all procedures were approved by the University of Pittsburgh Institutional Review Board.

#### MRI Method

MRI data were collected on a Siemens 3T magnet. A resting scan used a pulsed ASL sequence to obtain quantitative measures of CBF. Images were preprocessed to control for movement, to align scans, and to normalize to a standard brain space. Previously established analytic software\(^3\)–\(^6\) adjusted for our scan parameters assessed CBF. The online-only Data Supplement provides details on the MR technique and processing.

#### Metabolic Risk

Both the presence or absence of the MetS as well as a quantitative index of CMR was defined. According to National Cholesterol Education Program criteria,\(^7\) the MetS is defined as the presence of ≥3 of the following: (1) serum triglycerides ≥150 mg/dL; (2) fasting serum glucose ≥100 mg/dL; (3) waist circumference ≥102 cm in men or ≥88 cm in women; (4) systolic BP ≥130 or diastolic BP ≥85 mm Hg; (5) serum high-density lipoprotein cholesterol <40 mg/dL in men or <50 mg/dL in women. The scope of our study did not permit assessment of important possible pathologic correlates of MetS, for example, hepatic steatosis.

A composite index of CMR was calculated from the following criteria that define the MetS\(^9\): BP, waist circumference, high-density lipoprotein cholesterol, triglycerides, and glucose. Use of CMR as a continuous measure better predicts future cardiovascular disease events\(^23\) and would be expected in middle-aged samples, such as the current one to be a better predictor than the use of threshold values to calculate the syndrome as present or absent. The 5 risk factors (using systolic BP as the BP factor) were each standardized, and high-density lipoprotein was multiplied by −1. The 5 measures were summed and labeled as CMR score. The Framingham risk score was available as an additional index of known risk. This score is an accumulation of points assigned separately by sex for age, total cholesterol, smoking status, high-density lipoprotein, and systolic BP.\(^22\)

#### Carotid and White Matter Hyperintensity Measurement

Carotid artery intima-media thickness (IMT) was assessed by duplex (B-mode) ultrasonography using an Acuson Antares scanner (Acuson-Siemens, Malvern, PA). Four locations spanning the interior and exterior carotid in right and left arteries were assessed, and mean IMT from these areas formed the primary dependent variable currently used (see further details in online-only Data Supplement). This measure was unavailable for the validation sample. This sample, although assessed white matter hyperintensities using a validated,
automated technique. Total voxel counts of those showing a white matter hyperintensity were analyzed.

Analysis
Descrptive bivariate correlations between CBF and risk were followed by multiple regression modeling. The modeling first adjusted for demographic influences on CBF, and the risk factor was added as a second step. At initial step, age, race, current smoking status, sex, and total brain volume were input. The second step added the CMR measure, MetS, or the Framingham index. Age and smoking status are included in the Framingham index; therefore, in the second step for the Framingham index, separate age and smoking indices were not included. This basic analysis was repeated in the validation sample. Depending on the availability of the measure in a sample, carotid IMT or white matter hyperintensity measures were also assessed in step 2.

Results
Participant characteristics are presented in Table 1 for the primary and validation samples. The samples are quite comparable with reasonably typical values for their age cohort. The validation sample differs somewhat in mean age and racial composition in addition to the use of the pseudocontinuous ASL measure.

Carotid IMT and Metabolic Risk Related to CBF
Males, those with relatively higher body mass index and those possessing the MetS, had lower CBF. Race and nicotine use were unrelated. Table I in the online-only Data Supplement details these results for categorical participant characteristics. Table 2 presents the bivariate correlations with participant characteristics as well as the relation to the components of the syndrome and related insulin and homeostatic model assessment indices. Table 2 also illustrates the similarity of CBF and regional flow correlations using regions selected to approximate the watershed areas for the primary cerebral arteries, that is, anterior cerebral artery (medial frontal, superior parietal, and cingulate areas), middle cerebral artery (frontal, temporal, and inferior parietal), and posterior cerebral artery (occipital and inferior temporal; note that the correlations based on brain regions are only available for the larger of the 2 samples that were combined).

The Figure presents the scatter diagrams showing the correlations of overall CBF with CMR and carotid IMT. The correlation with CMR accounts for 8% of the variance in CBF, whereas that with carotid IMT accounts for 4%.

Multivariate Relations: CBF, IMT, and CMR
Table 3 shows the results for the basic model and for the models adding risk factors. Each risk factor was added individually to assess its specific relationship to CBF as a second step. Parallel models are presented for the basic sample as well as the validation sample. Separate metabolic variables were not separately tested but subsumed within the CMR and MetS variables.

In the initial model, age and sex showed directionally consistent influences on CBF, but these factors were not significant in the smaller validation sample. Brain size, smoking, and race did not show significant relationships in either sample.
Furthermore, CBF was independently related to carotid IMT and Framingham risk in the primary sample. The degree of relationship between CBF and vascular risk, although modest, is approximately half of the risk computed in this sample between being male or overweight/obese and carotid IMT, both accepted vascular risk factors. Our results are largely consistent with earlier work in older samples that has suggested decrements in CBF with age and vascular risk.15

Our cross-sectional observations do not allow any causal inferences. Metabolic factors could induce reductions in CBF, or cerebrovascular changes could precede metabolic changes. Longitudinal observations might determine whether CBF is modified before, after, or in conjunction with increases in risk via MetS factors. Relationships of CBF to risk were generally consistent across samples despite variation in ASL imaging; the variation although limits inferences about factors not related consistently across the primary and validation samples. This as well as sampling variability may contribute to the seemingly stronger relationship between CBF and MetS components in the smaller, validation sample. Although carotid IMT and Framingham risk were independently related to CBF in our primary sample, the relationship with metabolic factors was most consistent. CBF was also related significantly and similarly to each of the factors composing the MetS. Microcirculatory change more closely related to metabolic factors, such as hyperglycemia, is known to influence the structure and function of larger vessels, potentially altering large vessel flow, that is, CBF.31 Factors that we did not measure may, of course, have pathogenic influences on CBF and cardiometabolic disease, but the current concomitance of CBF and MetS factors argues for initially understanding this linkage. Our previous work has suggested that hypertension, a factor of the MetS, may have early effects on the brain that are not readily reversed by successful pharmacological treatment of hypertension.32 Examining metabolic factors taken together and atherosclerosis seems, however, to be equally or more important.

Reductions in CBF in conjunction with vascular risk might signal early atherosclerotic influences on the brain vasculature. Given the absence of regional specificity in our results, systemic factors should be considered. CBF varies as a function of the number of neurons and their overall activity, most primarily the energetic demand of postsynaptic potential reversal.33,34 For example, early atherogenic and vasoconstrictive effects of likely pathogenetic excesses of angiotensin II may impact CBF by reducing metabolic demand through neuronal cell death as well as tonically constricting the vasculature.19,35–37 The latter effect may impact cerebral autoregulation of blood flow,38 that is, challenge regulatory capacity. We examined white matter hyperintensities as a possible indicator of cortical arteriosclerosis.39 White matter hyperintensities were not related to CBF in the validation study, although this is not definitive, given the relatively young mean age of this sample. As noted previously, CBF has been related to white matter lesions in older samples,14,15 although ASL-assessed CBF was unrelated to Framingham risk in a small sample of older adults (mean age, 71.2; n=33, 15 with mild cognitive deficit).18 Of greater relevance, white matter disruption in a middle-aged sample with high BP was recently reported using diffusion tensor imaging to detect damage in white matter.

Adding CMR to the model demonstrated significant relationships with CBF in both the primary and validation samples. The relationship is modest, but consistent across samples. The dichotomized MetS variable relates to CBF significantly in the validation sample and is only directionally consistent with this in the primary sample. The Framingham risk score in contrast was significantly related to CBF in the primary sample, but only directionally consistent in the validation sample. Carotid IMT was available only in the primary sample, in which it was related significantly to CBF. Importantly, in the validation sample, automated voxel counts of white matter hyperintensities were available, but these were unrelated to CBF.

Discussion

The current results establish that CBF is related to CMR in midlife. The relationship is consistent, although modest, across the primary sample and the validation sample. CBF seems closely related to each of the components of the MetS, a known risk for both diabetes mellitus and cardiovascular disease.21,22 Furthermore, CBF was independently related to carotid IMT and Framingham risk in the primary sample. The degree of relationship between CBF and vascular risk, although modest, is approximately half of the risk computed in this sample between being male or overweight/obese and carotid IMT, both accepted vascular risk factors. Our results are largely consistent with earlier work in older samples that has suggested decrements in CBF with age and vascular risk.15

Our cross-sectional observations do not allow any causal inferences. Metabolic factors could induce reductions in CBF, or cerebrovascular changes could precede metabolic changes. Longitudinal observations might determine whether CBF is modified before, after, or in conjunction with increases in risk via MetS factors. Relationships of CBF to risk were generally consistent across samples despite variation in ASL imaging; the variation although limits inferences about factors not related consistently across the primary and validation samples. This as well as sampling variability may contribute to the seemingly stronger relationship between CBF and MetS components in the smaller, validation sample. Although carotid IMT and Framingham risk were independently related to CBF in our primary sample, the relationship with metabolic factors was most consistent. CBF was also related significantly and similarly to each of the factors composing the MetS. Microcirculatory change more closely related to metabolic factors, such as hyperglycemia, is known to influence the structure and function of larger vessels, potentially altering large vessel flow, that is, CBF.31 Factors that we did not measure may, of course, have pathogenic influences on CBF and cardiometabolic disease, but the current concomitance of CBF and MetS factors argues for initially understanding this linkage. Our previous work has suggested that hypertension, a factor of the MetS, may have early effects on the brain that are not readily reversed by successful pharmacological treatment of hypertension.32 Examining metabolic factors taken together and atherosclerosis seems, however, to be equally or more important.

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tracts (a relationship not present for white matter hyperintensities). Others have suggested that the MetS impairs brain capillary vasodilation through insulin resistance. Our data cannot specifically test this mechanism. In short, although reasonable alternatives exist, specific pathophysiological processes related to cardiovascular disease in the samples examined could not be identified, the mechanism underlying the observed relationship remains unknown.

Conclusions

Overall, the results confirm early observations that suggested that CBF might be reduced with age and vascular risk. The availability of an MRI sequence suitable for quantifying CBF made it possible to confirm these observations. The modest but consistent relationship of CMR to CBF is supportive of the involvement of cerebrovascular factors in risk at midlife. However, we have not been able to draw any specific implications of differences in CBF for cortical function. Finding the relationship at midlife in metabolic risk factors, likely themselves mediated by modifiable health behaviors, does strengthen the potential value of preventive measures taken well before old age. Progression of CBF changes with age may be of particular importance given observations of regional CBF relationships with preclinical indicators of vascular disease at older ages. Further examination of central regulation disruption as a component of CMR seems required.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

TOTAL CEREBRAL BLOOD FLOW RELATES TO KNOWN VASCULAR RISK FACTORS

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SUPPLEMENTAL METHODS

Inclusion Criteria. Participants for both projects were recruited from the greater Pittsburgh area through mailing designed to reach those eligible based on age and employment. Inclusion criteria were: 30-55 years of age, without: history of atherosclerotic disease (e.g., myocardial infarction) or treatment of such [e.g., angioplasty]; angina or claudication [by Rose questionnaire]; schizophrenia or bipolar disorder, chronic hepatitis, renal failure, neurological disorder, lung disease requiring drug treatment, stage 2 hypertension by screening blood pressure (BP) (SBP/DBP ≥160/100), alcohol consumption ≥5 portions 3-4 times/week; and in women, pregnancy. Persons prescribed any cardiovascular, psychotropic, glucocorticoid, lipid-lowering, diabetic or weight-loss medications were also excluded. Additional subject exclusions included: < 8th grade reading skills and shift workers.

MRI Data Collection. Neuroimaging data were acquired on a 3T Trio TIM whole-body scanner (Siemens, Erlangen, Germany), equipped with a 12-channel phased-array head coil. Resting perfusion images were acquired with a pulsed arterial spin-labeling (PASL) sequence.
For this sequence, interleaved perfusion images with and without arterial spin labeling were obtained over a 5 min 28 sec period using gradient-echo echo-planar imaging (EPI). The PASL sequence employed a modified version of the flow-sensitive alternating inversion recovery method, specifically applying a saturation pulse 700 ms after an inversion pulse. To reduce transit artifact, a 1000 ms delay separated the end of the labeling pulse and the time of image acquisition. Resting perfusion image acquisition parameters were: field of view (FOV) = 240×240 mm, matrix = 64×64, repetition time (TR) = 4000 ms, echo time (TE) = 18 ms, and flip angle (FA) = 90°. Twenty-one slices (5 mm thick, 1 mm gap) were acquired sequentially in an inferior-to-superior direction, yielding 80 total perfusion images (40 labeled, 40 unlabeled, 2 initial discarded images allowing for magnetic equilibration), and the acquisition time of each slice was 45 ms. A 24 sec of equilibrium magnetization of brain (two-sets of twenty-one slices; TR = 8000 ms; all other parameters are described as above) provided two images for CBF baseline quantification.

For spatial co-registration of resting perfusion images, T₁-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) neuroanatomical images were acquired over 7 min 17 sec by these parameters: FOV = 256×208 mm, matrix = 256×208, TR = 2100 ms, inversion time (TI) = 1100 ms, TE = 3.29 ms, and FA = 8° (192 slices, 1mm thick, no gap).

For ancillary analyses addressing the impact of total brain volume on CBF related findings, we used the Oxford University Centre for Functional MRI of the Brain (FMRIB) Integrated Registration and Segmentation Tool (FIRST) in the FMRIB Software Library (FSL) version 4.0. FIRST is a semi-automated model-based image segmentation tool that relies on a Bayesian framework, as well as shape and appearance models obtained from manually segmented images provided by the Center for Morphometric Analysis, Massachusetts General
Hospital (Boston, MA). Volumetric measures in mm$^3$ were generated from our T$_1$-weighted images (for a more detailed description of this method, see).\textsuperscript{2,3}

**Preprocessing of neuroimaging data.** Resting perfusion images were preprocessed with computational routines implemented in Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK). For preprocessing, MPRAGE was segmented into grey and white images. Resting perfusion images were realigned to the first image of the series. The baseline images were realigned to the first of the perfusion images, and one averaged baseline image was then calculated from the two realigned images for later use of CBF imaging reconstruction. Each individual’s grey image realigned was co-registered to the respective mean perfusion image. The 80 realigned perfusion images and one averaged baseline image were then smoothed with a 12mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. The already realigned and smoothed 40 labeled and 40 unlabeled perfusion images were submitted to pair-wise subtraction. Subtraction images were converted to an absolute CBF image series using a validated algorithm.\textsuperscript{4} This perfusion series was then averaged, generating for each individual a single resting voxel-wise CBF image and a global CBF value, both in units of mL/100g/min. After the perfusion reconstruction step, individual’s mean CBF images and structural grey image were then spatially normalized to the International Consortium for Brain Mapping 152 template (Montreal Neurological Institute; MNI) of grey image with voxel size of 3x3x3 mm to preserve concentration of the image using trilinear interpolation method. A mean CBF image was then generated for each participant.

Brain image data collection and processing were essentially similar for the generalization sample except for the use of a different ASL sequence. The generalization sample used pseudocontinuous ASL and closely followed the collection and preprocessing procedures.
developed by Detre, Wang and colleagues.\textsuperscript{5,6}

**Carotid IMT Assessment.** B-mode images were obtained from the following 4 locations of the left and right carotid arteries: the near and far walls of the distal common carotid artery, 1cm proximal to the carotid bulb; the far walls of the carotid bulb, from the point where the near and far walls are no longer parallel and extending down to the flow divider; and the internal carotid artery from the flow divider to 1cm distal from the flow divider. For these regions, an image was digitized for automated scoring by edge detection software (Artery Measurement System; Goteborg University, Sweden). The software generates two lines: one along the lumen-intima interface and one along the media-adventitia interface. The distances between the interfaces are measured in 1cm increments, generating one measurement (in mm) per pixel in each region (~140 total). Mean IMT is computed as the average of all intima-media distance values in mm in both carotid arteries. All readings were conducted by trained technicians at the University of Pittsburgh Ultrasound Research Laboratory. Replicate readings were performed by different technicians on 41 ultrasound scans obtained from 2008-2011 to assess the inter-reader reproducibility of IMT measures. Intra-class correlation was 0.88 for IMT. Reproducibility has been previously reported as well.\textsuperscript{7,8}

**Supplemental Table I. Differences of Mean CBF according to Subject Characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Means</th>
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<th>Probability</th>
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<td>Male-Female</td>
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<tr>
<td>&lt;26 vs&gt;=26</td>
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Watershed Areas. In order to assess possible regional specificity and, more particularly, a vascular specificity to the CBF results, we selected regions of interest from the Wake Forest Pick Atlas that corresponded to the circulation of the major cerebral arteries.\textsuperscript{9,10} CBF from the selected regions was then combined and related to variables comprising the MetS as shown in the primary paper. Mean CBF for the regions and the regions of interest included (Labels) are shown below.

**Anterior Cerebral Artery**: Medial Frontal Gyrus, Superior Parietal Lobule, Anterior Cingulate, Cingulate Gyrus

**Middle Cerebral Artery**: Inferior Parietal Lobule, Middle Frontal Gyrus, Superior Temporal Gyrus, Middle Temporal Gyrus, Inferior Frontal Gyrus

**Posterior Cerebral Artery**: Inferior Temporal Gyrus, Superior Occipital, Middle Occipital, Inferior Occipital

SUPPLEMENTAL REFERENCES

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