Increased Microvascularization and Vessel Permeability Associate With Active Inflammation in Human Atheromata

Viviany R. Taqueti, MD, MPH; Marcelo F. Di Carli, MD; Michael Jerosch-Herold, PhD; Galina K. Sukhova, PhD; Venkatesh L. Murthy, MD, PhD; Eduardo J. Folco, PhD; Raymond Y. Kwong, MD, MPH; C. Keith Ozaki, MD; Michael Belkin, MD; Matthias Nahrendorf, MD, PhD; Ralph Weissleder, MD, PhD; Peter Libby, MD

Background—Studies have shown the feasibility of imaging plaques with 2-deoxy-2-\[^{18}\text{F}\]fluoroglucose (FDG) positron emission tomography and dynamic contrast–enhanced magnetic resonance imaging with inconsistent results. We sought to investigate the relationship between markers of inflammatory activation, plaque microvascularization, and vessel wall permeability in subjects with carotid plaques using a multimodality approach combining FDG positron emission tomography, dynamic contrast–enhanced magnetic resonance imaging, and histopathology.

Methods and Results—Thirty-two subjects with carotid stenoses underwent noninvasive imaging with FDG positron emission tomography and dynamic contrast–enhanced magnetic resonance imaging, 46.9% (n=15) before carotid endarterectomy. We measured FDG uptake (target:background ratio [TBR]) by positron emission tomography and K\text{trans} (reflecting microvascular permeability and perfusion) by magnetic resonance imaging and correlated imaging with immunohistochemical markers of macrophage content (CD68), activated inflammatory cells (major histocompatibility complex class II), and microvessels (CD31) in plaque and control regions. TBR and K\text{trans} correlated significantly with tertiles of CD68+ (P=0.009 and P=0.008, respectively), major histocompatibility complex class II+ (P=0.003 and P<0.001, respectively), and CD31+ (P=0.004 and P=0.008, respectively). Regions of plaques were associated with increased CD68+ (P=0.002), major histocompatibility complex class II+ (P=0.002), CD31+ (P=0.02), TBR (P<0.0001), and K\text{trans} (P<0.0001), as compared with those without plaques. Microvascularization correlated with macrophage content (r=0.52; P=0.007) and inflammatory activity (r=0.68; P=0.0001) and TBR correlated with K\text{trans} (r=0.53; P<0.0001). In multivariable mixed linear regression modeling, TBR remained independently associated with K\text{trans} ([β(SE], 2.68[0.47]; P<0.0001).

Conclusions—Plaque regions with active inflammation, as determined by macrophage content and major histocompatibility complex class II expression, showed increased FDG uptake, which correlated with increased K\text{trans} and microvascularization. The correlation between K\text{trans} and TBR was moderate, direct, highly significant, and independent of clinical symptoms and plaque luminal severity. (Circ Cardiovasc Imaging. 2014;7:920-929.)

Key Words: atherosclerotic plaque ■ inflammation ■ molecular imaging ■ neovascularization

Inflammatory signaling mediated by activated macrophages contributes to the formation of atherosclerotic plaques with characteristics associated with fatal thrombosis, and inflammation associates with incident cardiovascular events. Inflammation provides a mechanistic link between traditional cardiovascular risk factors such as hypertension and low-density lipoprotein, and the altered biological responses of the artery wall that drive atherosclerosis and its complications. Neovascularization of the intima accompanies inflammation and atherogenesis. Friable plaque microvessels, with the potential to promote intraplaque hemorrhage, thrombosis in situ, lipid-rich necrotic core accumulation, and subsequent plaque expansion, likely contribute to clinical complications.

Clinical Perspective on p 929

In human carotid disease, current diagnostic and therapeutic guidelines emphasize symptoms and luminal stenosis severity in patient selection for invasive revascularization, yet these indices may lack sensitivity and specificity for predicting optimally the risk of future atherothrombotic events. Molecular and pharmacokinetic imaging techniques, such as 2-deoxy-2-\[^{18}\text{F}\]fluoroglucose (FDG) positron emission tomography and dynamic contrast–enhanced magnetic resonance imaging, allow for in-house assessment of plaque composition and inflammation, as well as the identification of vulnerable plaques that are predictive of adverse events. Molecular and pharmacokinetic imaging techniques, such as 2-deoxy-2-\[^{18}\text{F}\]fluoroglucose (FDG) positron emission tomography and dynamic contrast–enhanced magnetic resonance imaging, allow for in-house assessment of plaque composition and inflammation, as well as the identification of vulnerable plaques that are predictive of adverse events.
tomography/computed tomography (PET/CT) and dynamic contrast–enhanced magnetic resonance imaging (DCE-MRI), allow measurement of metabolic activity and neovascularization, respectively. Although studies have shown the feasibility of imaging plaques with FDG-PET and DCE-MRI, and some have attempted limited correlation between histological staining and PET or MRI, inconsistent results have emerged, perhaps underscoring that the mere presence of macrophages or microvessels may not reflect the prevailing inflammatory activity. Indeed, mononuclear phagocytes exhibit considerable functional diversity, such that simply enumerating these cells discloses little about their state of inflammatory activation.

We thus sought to investigate the relationship between markers of inflammatory activation, plaque microvascularization, and vessel wall permeability in patients with carotid plaques, using a multimodality approach combining FDG-PET, DCE-MRI, and histopathology. This study tested the hypotheses that (1) FDG uptake by PET and (2) microvascular permeability and perfusion by DCE-MRI correlate with histological markers of macrophage content, active inflammation, and plaque microvascularization, and (3) FDG uptake correlates with microvascular permeability, independently of anatomic stenosis severity.

Methods

Study Population

This study enrolled prospectively 32 subjects who presented electively for evaluation of carotid stenoses and underwent imaging with FDG PET/CT and DCE-MRI before possible carotid endarterectomy (CEA) at Brigham and Women’s Hospital (Figure 1A). Before enrollment, carotid stenoses were assessed by Doppler ultrasonography as clinically indicated. Exclusion criteria included contraindications to PET/CT or MRI, including pregnancy or lactating state, renal dysfunction (estimated glomerular filtration rate <60 mL/min), hemodynamic instability, presence of metallic implants, and significant claustrophobia. Clinical history and medication use were ascertained at the time of subject enrollment. After obtaining written informed consent, study participants underwent carotid PET/CT and MRI (Figure 1B). In those scheduled to undergo CEA as part of standard clinical care, imaging occurred within 14 days before endarterectomy, at which time excised plaques were removed intact for histological analysis. Blood samples for biomarkers were collected just before CEA or before imaging if no CEA was planned. The study was approved by the Partners Healthcare Institutional Review Board and conducted in accordance with institutional guidelines.

![Figure 1. A. Schematic of study procedures. *Surgical denotes patients who underwent carotid endarterectomy (CEA) for obstructive plaque in the internal carotid artery (ICA) or bifurcation of the common carotid artery (CCA). †FDG PET/CT denotes 2-deoxy-2-[18F]fluoroglucose positron emission tomography/computed tomography imaging; ‡DCE-MRI denotes dynamic contrast–enhanced magnetic resonance imaging; and **analyzable control denotes analyzable control regions remote from plaque. Each subject had the potential of contributing up to 1 plaque and 1 control region per carotid artery. For FDG PET/CT and DCE-MRI, bilateral imaging yielded up to 2 plaques and 2 control regions per subject (not all were analyzable). B, Protocol timeline. C, Imaging of the carotid bifurcation for plaque colocalization. Identification of plaque relative to the carotid bifurcation allowed for colocalization of PET/CT and MRI images with carotid endarterectomy specimens. A representative MRI screenshot from a subject with a right internal carotid plaque (arrowhead) is shown. Control regions were defined >1 cm away from plaques.](http://circimaging.ahajournals.org/)}
PET/CT Imaging

Patients were imaged with a whole-body PET/CT scanner (Discovery STE LightSpeed 64, GE Healthcare, Milwaukee, WI) with a 15-cm field of view that generated 47 image planes with a slice thickness of 3.2 mm. A rigid, MR-compatible head holder with fixation straps was used to minimize involuntary motion of the head and neck. All patients had a blood glucose concentration <200 mg/dL at the time of imaging, and long-acting medications, except for insulin, were omitted on the morning of imaging; any subjects on long-acting insulin were instructed to take half of their usual dose. After an overnight fast, patients were injected intravenously with 10 mCi of FDG. After a distribution period of 90 minutes, a dedicated head–neck PET imaging in 3-dimensional (3D) list mode was performed in 1 bed position for 20 minutes (matrix 128×128). Low-dose, noncontrast helical CT imaging (140 kV, 20 mA) was performed over the same range, from the skull base to 3 cm below the level of the carotid bifurcation, for attenuation correction of PET images and anatomic localization of FDG uptake. Radiation exposure per study was <8 mSv.

Measuring FDG Uptake and TBR

FDG PET/CT images were analyzed as previously described. Carotid FDG uptake was measured at 3- to 4-mm intervals along the length of the carotid artery by an experienced observer blinded to the MRI and histopathology results. Regions of interest were drawn in the internal carotid artery or common carotid artery bifurcation using minimum area boundaries at slice locations matching the MRI-defined plaque or control regions, using the CT scan coregistered to FDG-PET images. For each slice, standardized uptake values (SUVmax, SUVmean) were measured as the maximum and mean pixel activity within the regions of interest, respectively, using an approach standardized to body weight (BW, in g): SUV = tissue activity (μCi/mL)/injected activity per BW (μCi/g). Three measurements of SUV were recorded along contiguous slices of plaque or control region to produce an average whole arterial SUV Per region. Finally, the whole arterial SUV was corrected for blood activity by dividing by the average blood SUV obtained from 3 regions of interest from the internal jugular vein, to produce a blood-corrected arterial wall SUV correction factor. SUVmean or target/background ratio (TBR). Maximum TBR (TBRmax) and mean TBR (TBRmean) correspond to values derived from SUVmax and SUVmean, respectively.

Dynamic Contrast–Enhanced MRI

Dynamic contrast–enhanced MRI was performed on a 3-Tesla whole-body scanner (Tim Trio, Siemens Medical Solutions, Erlangen, Germany) with a built-in transmit body coil, with high-performance gradients (maximum amplitude of 40 mT/m and slew rate of 200 mT/m/s), and parallel image acquisition capabilities. Custom-built, receive-only, dual-phased arrays with a total of 8 receive channels were used for parallel bilateral carotid imaging; each array consisted of 4 loops with 4.8 cm diameter and 2 cm overlap. To minimize motion, subjects were positioned in a foam head holder with a saturation pad placed over the anterior neck to reduce head mobility and minimize the air–tissue susceptibility interface below the jaw line. The examination included 3D T2-weighted turbo-spin echo sequences with high sampling efficiency (Sampling Perfection with Application Optimized Contrasts using Different Flip Angle Evolutions, SPACE). Nonselective refocusing pulses with variable flip angles tailored to a prescribed signal evolution were applied for localization and definition of carotid bifurcation anatomy and visualization of carotid plaque (repetition time/echo time, 1300/119 ms; echo-train length, 51; isotropic resolution, 0.8 mm; integrated parallel imaging acceleration factor, 2). In the 3D SPACE acquisitions, the read-out direction was oriented along the carotid vessel axis to suppress signal from flowing blood. The protocol for T1-weighted dynamic imaging of carotid wall contrast enhancement was based on a 3D fast-gradient-echo technique (TR/TE/flip, 4.3/2.3/20°), with an acquisition time per dynamic view of 10 seconds, using a parallel imaging acceleration factor of 2 (with 24 reference lines). A total of 20 dynamic views were obtained for a slab thickness of 5 cm, phase-encodings giving a slice thickness of 3 mm, and an isotropic in-plane resolution of 0.7 mm. A gadolinium-based (Gd) contrast agent (Magnevist, Berlex, Wayne, NJ) was injected at the end of the second dynamic sequence at a concentration of 0.1 mmol Gd/kg and a rate of 2 mL/s via a power injector, so as to be coincident with acquisition of the second image in the sequence. After acquisition, plaques at the level of the internal carotid artery or common carotid bifurcation bilaterally were identified for analysis, with control regions defined as >1 cm away from plaques.

Measuring Ktrans

Inner and outer vessel wall contours were manually traced on all slices corresponding to plaque and control regions by an experienced observer blinded to the PET/CT and histopathology results. Contrast agent dynamics for the region between contours were quantified as previously described using a Kety–Schmidt kinetic model for dynamic contrast-enhanced MRI. This model assumes that contrast agent concentration is proportional to signal intensity change, and that reflux of contrast agent from the plaque to plasma is negligible after studies of short duration. The average value of Ktrans (volume transfer constant from plasma to extravascular extracellular tissue compartment) over the entire plaque or control region was determined. Image analysis and kinetic modeling were integrated into an in-house program with graphical user interface implemented in the Matlab environment (The Mathworks, Natick, MA).

Plaque Histopathology

Excised specimens from subjects after CEA underwent histopathologic and immunohistochemical examination as previously described, using protocols approved by the Partners Human Research Committee at Brigham and Women’s Hospital. Specimens were cut in 4-mm rings grouped by distance relative to the carotid bifurcation, embedded, and sectioned before immunohistochemical analysis with monoclonal antibodies against CD68 for macrophage content, major histocompatibility complex class II (MHC-II) for activated inflammatory cells, and CD31 for microvessels (Dako North America, Inc). An experienced observer blinded to the PET/CT and DCE-MRI results performed the analyses. Measurement of CD68 and MHC-II staining used computer-assisted color image analysis to determine percent positive areas, and microvessel profiles identified by CD31 staining were quantified as number of microvessels per millimeter squared.

Colocalization Between FDG PET/CT, DCE-MRI, and Histopathology

Anatomic colocalization between corresponding PET/CT and DCE-MRI slices (and histopathologic sections, if available) was performed by locating plaques and control regions relative to the carotid bifurcation on the left and right sides of each subject. The carotid bifurcation was defined as the apex of the luminal flow divider between the internal and external carotid arteries (Figure 1C), as identified on MRI and CT, or in excised specimens. Locations of plaques and control regions on the MRI data set were recorded along axial images at 5-mm intervals and referenced according to the inferosuperior distance from the flow divider. PET/CT and histological data matching corresponding locations on MRI were obtained, accounting for an expected 25% contraction in tissue length after histological processing. For subjects without MRI data, locations of plaques and control regions were defined histologically and matched to PET/CT. Matched cross-sections were compared quantitatively for FDG TBR, Ktrans, and immunohistochemical markers.

Statistical Analysis

Baseline characteristics are reported as rates with percentages (%) for categorical variables and medians with interquartile ranges for continuous variables. We used Fisher exact test and the Wilcoxon rank-sum test to assess differences in categorical and continuous baseline characteristics. Because of modest kurtosis in the distribution of some histological and noninvasive imaging markers, values were natural log transformed before using Pearson correlation to describe the
association between continuous variables. To account for any correlation between measurements (plaque and control region) within each subject who underwent carotid endarterectomy, we repeated the analysis using a mixed linear model with an unstructured covariance matrix. Doing so did not significantly alter results, as there was no significant correlation between plaque and control regions within subjects. Recognizing that the associations between histological and noninvasive imaging markers may not follow strictly linear relationships, we also analyzed values of FDG TBR and K<sub>trans</sub> by tertiles of histological markers, with comparisons between tertiles based on the Kruskal–Wallis test. Similar results were obtained for TBR<sub>mean</sub> and TBR<sub>max</sub>, and only TBR<sub>mean</sub> is shown for simplicity, consistent with prior publications.<sup>8</sup>

Regions of plaque and control were analyzed in pairwise fashion for the staining of histological markers, uptake of FDG TBR, and kinetic modeling of K<sub>trans</sub> using the paired Wilcoxon signed-rank test. To depict the association between markers of inflammation and microvascularization, we used Spearman correlation, rather than Pearson correlation with logarithmic transformation, to allow for ready interpretation of values on accompanying scatter plots. To account for

### Table 1. Baseline Characteristics of Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=32)</th>
<th>Surgical* (n=15)</th>
<th>Nonsurgical (n=17)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age,‡ y (IQR)</td>
<td>68 (65–76)</td>
<td>73 (66–76)</td>
<td>66 (63–75)</td>
<td>0.15</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>16 (50.0)</td>
<td>9 (60.0)</td>
<td>7 (41.2)</td>
<td>0.48</td>
</tr>
<tr>
<td>White race (%)</td>
<td>31 (96.9)</td>
<td>14 (93.3)</td>
<td>17 (100)</td>
<td>0.47</td>
</tr>
<tr>
<td>Prior medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23 (71.9)</td>
<td>14 (93.3)</td>
<td>9 (52.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>26 (81.3)</td>
<td>12 (80.0)</td>
<td>14 (82.4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>10 (31.3)</td>
<td>5 (33.3)</td>
<td>5 (29.4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Prior smoker (%)</td>
<td>24 (75.0)</td>
<td>9 (60.0)</td>
<td>15 (88.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>11 (34.4)</td>
<td>4 (26.7)</td>
<td>7 (41.2)</td>
<td>0.47</td>
</tr>
<tr>
<td>Peripheral arterial disease (%)</td>
<td>19 (59.4)</td>
<td>8 (53.3)</td>
<td>11 (64.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>Stroke or transient ischemic attack (%)</td>
<td>5 (15.6)</td>
<td>0 (0)</td>
<td>5 (29.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Concurrent neurological symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient ischemic attack or amaurosis fugax, %</td>
<td>4 (12.5)</td>
<td>4 (26.7)</td>
<td>0 (0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Medications§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>30 (93.8)</td>
<td>14 (93.3)</td>
<td>16 (94.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>10 (31.3)</td>
<td>4 (26.7)</td>
<td>6 (35.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>29 (90.6)</td>
<td>14 (93.3)</td>
<td>15 (88.2)</td>
<td>0.99</td>
</tr>
<tr>
<td>β-blocker (%)</td>
<td>18 (56.3)</td>
<td>7 (46.7)</td>
<td>11 (64.7)</td>
<td>0.48</td>
</tr>
<tr>
<td>Angiotensin inhibitor (%)</td>
<td>18 (56.3)</td>
<td>10 (66.7)</td>
<td>8 (47.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Laboratory values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol,‡ mg/dL</td>
<td>151 (139–172)</td>
<td>166 (140–192)</td>
<td>147 (134–164)</td>
<td>0.26</td>
</tr>
<tr>
<td>Triglycerides,‡ mg/dL</td>
<td>98 (57–125)</td>
<td>104 (77–142)</td>
<td>78 (52–102)</td>
<td>0.25</td>
</tr>
<tr>
<td>High-density lipoprotein,‡ mg/dL</td>
<td>46 (37–61)</td>
<td>42 (37–61)</td>
<td>50 (40–61)</td>
<td>0.50</td>
</tr>
<tr>
<td>Low-density lipoprotein,‡ mg/dL</td>
<td>81 (69–104)</td>
<td>104 (72–114)</td>
<td>76 (67–85)</td>
<td>0.17</td>
</tr>
<tr>
<td>Glucose,‡ mg/dL</td>
<td>99 (94–106)</td>
<td>99 (94–106)</td>
<td>100 (95–108)</td>
<td>0.79</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein,‡ mg/L</td>
<td>0.86 (0.41–1.94)</td>
<td>0.73 (0.41–2.00)</td>
<td>1.05 (0.41–1.80)</td>
<td>0.81</td>
</tr>
<tr>
<td>Interleukin,‡ pg/mL</td>
<td>2.4 (1.5–3.1)</td>
<td>2.3 (1.5–3.0)</td>
<td>2.4 (1.5–3.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>CD40 ligand,‡ pg/mL</td>
<td>652 (238–1170)</td>
<td>792 (260–2049)</td>
<td>645 (217–1088)</td>
<td>0.44</td>
</tr>
<tr>
<td>Tumor necrosis factor receptor type II,‡ ng/mL</td>
<td>2.6 (1.9–2.9)</td>
<td>2.5 (1.8–3.0)</td>
<td>2.6 (1.9–2.8)</td>
<td>0.99</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1,‡ ng/mL</td>
<td>227 (199–282)</td>
<td>236 (210–277)</td>
<td>228 (188–286)</td>
<td>0.61</td>
</tr>
<tr>
<td>Vascular cell adhesion molecule 1,‡ ng/mL</td>
<td>801 (588–1014)</td>
<td>768 (668–888)</td>
<td>846 (562–1075)</td>
<td>0.74</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1,‡ ng/mL</td>
<td>2.7 (2.0–3.9)</td>
<td>2.6 (2.0–5.1)</td>
<td>3.1 (2.0–3.8)</td>
<td>0.90</td>
</tr>
<tr>
<td>Carotid artery Doppler ultrasonography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doppler flow velocity at plaque,‡ cm/s</td>
<td>310 (210–363)</td>
<td>363 (355–425)</td>
<td>215 (202–257)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque‡ stenosis, %</td>
<td>75 (60–80)</td>
<td>80 (78–88)</td>
<td>63 (58–68)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range.

*Surgeon denotes subjects who underwent carotid endarterectomy.
†The P value is for the comparison between groups and is based on the Fisher exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables.
‡Continuous variables are presented as medians (interquartile ranges).
§Medications taken for ≥4 weeks before testing.
||Plaque at internal carotid artery or bifurcation of common carotid artery.
the existence of bilateral noninvasive imaging data points in some subjects, the analysis was repeated using a mixed linear model, but results were not significantly different.

Finally, mixed linear regression models were used to determine the factors most strongly associated with mean K<sub>trans</sub> values after natural logarithmic transformation. Candidate variables tested included demographic characteristics, medical history, and medication use, with the most significant and clinically important univariable associations included in the multivariable model. A second model added laboratory values as covariates, with only the most significant associations included in the final multivariable model. In this way, the first multivariable model was adjusted for TBR<sub>mean</sub> (natural log transformed), age, sex, presence of hypertension or diabetes mellitus, use of statin medication, neurological symptoms, and plaque stenosis >70%. The second multivariable model was adjusted for all of these factors, as well as serum levels of interleukin-6 and tumor necrosis factor receptor II. Only variables showing significant associations in the models are displayed. A P value of <0.05 was considered to indicate statistical significance, and all tests were 2-sided. The SAS analysis system, version 9.3, was used for all analyses (SAS Institute).

Results

Baseline Characteristics

Distribution of baseline characteristics is shown between subjects who did and did not undergo carotid endarterectomy (Table 1). The median (interquartile range) age of subjects in the overall cohort was 68 (65–76) years, 50.0% were men, and most had a history of hypertension, dyslipidemia, and prior tobacco use. Compared with subjects without surgical intervention (n=17), those who underwent carotid endarterectomy (n=15) were more symptomatic (0 versus 26.7%; \( P=0.04 \)) and demonstrated carotid plaques of greater anatomic severity (median [interquartile range] percent stenosis, 63 (58–68) versus 80 (78–88), \( P<0.001 \), respectively), but with similar levels of serum markers of inflammation.

Markers of Inflammation and Microvascularization Colocalize to Plaque in the Human Carotid

Figure 1A delineates the resultant number of analyzable plaques and control regions remote from plaque in the overall cohort by imaging modality. Summary statistics for SUV<sub>BW</sub>, TBR, and K<sub>trans</sub> are shown in the Table in the Data Supplement. Representative images from a subject showing features of atherosclerotic plaque in the right internal carotid artery are shown in Figure 2. FDG uptake (TBR<sub>mean</sub>, 1.77; TBR<sub>max</sub>, 2.09) colocalized with microvascular volume transfer constant (K<sub>trans</sub>, 1.78/min), macrophage content (CD68<sup>+</sup>, 26.9%), active inflammation (MHC-II<sup>+</sup>, 21.3%), and microvascularization (CD31<sup>+</sup>, 4.30 microvessels/mm<sup>2</sup>) at the site of anatomic plaque.
Histological and Noninvasive Imaging Markers Correlate Highly

Significant, moderate to strong direct correlations were observed between immunohistochemical staining in excised specimens and noninvasive functional imaging markers (Table 2; Pearson correlations are shown between natural log–transformed values of markers). Findings were significant for both TBRmax and TBRmean. Of the histological markers, MHC-II, reflecting not only the presence but also the inflammatory activation of cells, showed the most robust correlations with FDG uptake by PET (Pearson r=0.66 and 0.63; P<0.001 for both, for TBRmax and TBRmean, respectively) and Ktrans by DCE-MRI (Pearson r=0.87; P<0.001). Furthermore, both FDG TBRmean and Ktrans associated significantly with tertiles of histological markers (Figure 3). Similar results were obtained for TBRmax (data not shown).

Histological and Noninvasive Imaging Markers Colocalize With Regions of Plaques

Relative to uninvolved regions, those with plaques showed significant associations with increased macrophage content, MHC-II expression, microvascularization, FDG uptake, and Ktrans (Figure 4).

![Image of Figure 3](http://circimaging.ahajournals.org/)

**Table 2. Correlations of Histological and Noninvasive Imaging Markers**

<table>
<thead>
<tr>
<th>Noninvasive Imaging Marker</th>
<th>FDG-PET (n=25)</th>
<th>DCE-MRI (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBRmax</td>
<td>TBRmean</td>
</tr>
<tr>
<td></td>
<td>r (95% CI)</td>
<td>r (95% CI)</td>
</tr>
<tr>
<td>Histological marker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68+</td>
<td>0.64 (0.32–0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MHC-II+</td>
<td>0.66 (0.35–0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD31+</td>
<td>0.54 (0.16–0.78)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; DCE-MRI, dynamic contrast–enhanced magnetic resonance imaging; FDG-PET, 2-deoxy-2-[18F]fluoroglucose positron emission tomography; and TBRmax and TBRmean denote maximum and mean target:background ratios for 2-deoxy-2-[18F]fluoroglucose uptake, respectively.

*The P value is for the Pearson correlation (r) between natural log–transformed values of markers.
Microvascularization and Vessel Wall Permeability Correlate With Inflammation

In immunohistochemical examination of excised endarterectomy specimens as well as noninvasive functional imaging of carotid arteries, markers of inflammation showed significant and moderate direct correlations with those of microvascularization (Figure 5A and 5B) and vessel permeability (Figure 5C). Specifically, the following correlations ($r$ [95% confidence interval]) were observed: for CD31 and CD68 ($r$=0.52 [0.15–0.75]; $P$=0.007), for CD31 and MHC-II ($r$=0.68 [0.37–0.84]; $P$=0.0001), and for $K_{\text{trans}}$ and FDG TBRmean ($r$=0.53 [0.37–0.66]; $P$<0.001). Scatter plots for CD31 versus CD68 or MHC-II staining, as well as for $K_{\text{trans}}$ versus FDG TBRmean, illustrate the range of correlated values for regions with and without plaques. Of note, there was no significant relationship between the presence of neurological symptoms and histological inflammation and microvascularization, nor between the presence of severe (>70%) anatomic stenosis and functional noninvasive markers of inflammation and microvascular permeability.

FDG Uptake Associates Independently With Microvascularization and Vessel Permeability in Human Atheroma

In univariable analysis, TBRmean associated directly with mean $K_{\text{trans}}$ ($\beta$ coefficient [SE] for natural log–transformed values, 2.47 [0.43]; $P$<0.0001; Table 3). There was also a significant inverse association between statin medication use and $K_{\text{trans}}$ ($\beta$ [SE] for natural log–transformed value, −1.07 [0.34]; $P$=0.004). Both the association between FDG TBRmean and $K_{\text{trans}}$ and that between statin use and $K_{\text{trans}}$ remained significant in a multivariable mixed linear regression model incorporating age, sex, presence of hypertension or diabetes mellitus, statin use, plaque severity >70%, and FDG TBRmean ($\beta$ [SE] for natural log–transformed values, 2.63 [0.45]; $P$<0.0001 and −1.17 [0.45]; $P$=0.02 for TBRmean and statin use, respectively; Table 3). After adjusting further for serum markers of inflammation, including interleukin-6 and tumor necrosis factor receptor II, only FDG TBRmean remained independently associated with mean $K_{\text{trans}}$ ($\beta$ [SE] for natural log–transformed values, 2.68 [0.47]; $P$<0.0001).

Discussion

This study demonstrated that plaque regions with active inflammation, as determined by macrophage content and MHC-II expression, show increased FDG uptake, microvascularization by CD31 immunoreactivity, and $K_{\text{trans}}$ consistent with heightened microvascular permeability. The coincidence of inflammation and microvascularization with elevated FDG uptake provides additional mechanistic insight into the interpretation of FDG signal in human plaques. These in vivo data bolster our previous in situ observations colocalizing macrophages, angiogenic growth factors, and microvessels in human atheromata.4 The correlation between $K_{\text{trans}}$ and FDG TBR was moderate, direct, and highly significant, and this association did not depend on other risk factors, including the presence of clinical symptoms, obstructive plaque, or serum biomarkers. The cohort studied included a range of plaque stenosis severity, in addition to control regions, in patients undergoing evaluation for carotid revascularization. Although all correlations between histological and noninvasive imaging markers were at least moderate in magnitude and nearly

Figure 4. Histological and noninvasive imaging markers colocalize with plaques. Regions of anatomic plaque were associated with increased numbers of macrophages (CD68*, $P$=0.002), activated cells (major histocompatibility complex II [MHC]-II*, $P$=0.002), microvascularization (CD31*), mean 2-deoxy-2-[18F]fluoroglucose uptake (mean target:background ratio [TBRmean], $P$=0.0001) and mean $K_{\text{trans}}$ ($P$<0.0001), as compared with regions without plaques. The $P$ value is for the comparison between groups and is based on the paired Wilcoxon signed-rank test.
all significant, the most robust correlations emerged between MHC-II, a marker of cells activated by the T-helper 1 cytokine interferon-γ, and the functional imaging markers of FDG TBR and K\textsubscript{trans}. As such, a novel aspect of this study is its focus on specific markers of inflammatory activation, rather than simply the enumeration of inflammatory cells. Although macrophages likely account for the bulk of the MHC-II expression (and FDG uptake) in the plaque, smooth muscle cells can also express MHC-II when stimulated by interferon-γ. Moreover, cytokine-stimulated smooth muscle cells augment glucose uptake. Thus, some of the increased FDG signal in atheromata may also derive from uptake by smooth muscle cells that have encountered T-helper 1 cytokines.

Previous studies have explored the relationship between FDG PET and DCE-MRI with inconsistent results. In 40 subjects with coronary heart disease risk equivalents (but

Table 3. Factors Most Strongly Associated With K\textsubscript{trans} in Mixed Linear Regression Models

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Univariable Model</th>
<th>Multivariable Model 1†</th>
<th>Multivariable Model 2‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>P Value</td>
<td>β (SE)</td>
</tr>
<tr>
<td>TBR\textsubscript{mean}§</td>
<td>2.47 (0.43)</td>
<td>&lt;0.0001</td>
<td>2.63 (0.45)</td>
</tr>
<tr>
<td>Statin</td>
<td>−1.07 (0.34)</td>
<td>0.004</td>
<td>−1.17 (0.45)</td>
</tr>
</tbody>
</table>

β: estimate with SE are listed for each linear regression model.

*K\textsubscript{trans} denotes natural log-transformed K\textsubscript{trans} values.

†Incorporating age, sex, presence of hypertension or diabetes mellitus, use of statin medication, neurological symptoms, plaque severity >70%, and TBR\textsubscript{mean}.

‡Incorporating age, sex, presence of hypertension or diabetes mellitus, use of statin medication, neurological symptoms, plaque severity >70%, TBR\textsubscript{mean}, and serum levels of interleukin-6 and tumor necrosis factor receptor II.

§TBR\textsubscript{mean} denotes natural log-transformed mean target:background ratio for 2-deoxy-2-[18F]fluoroglucose uptake.
not necessarily significant carotid stenoses) who were also on lipid-lowering therapy to achieve low-density lipoprotein cholesterol levels of <100 mg/dL, Calcagno et al. found a weak, inverse correlation between mean FDG TBR and mean Ki\textsuperscript{trans}, which lost statistical significance after correction for multiple testing. In contrast, Truijman et al recently reported a positive and significant weak correlation \((r=0.30; P=0.035)\) between FDG TBR and mean Ki\textsuperscript{trans} in 49 patients with carotid stenosis of 30% to 69% and transient ischemic attack or minor stroke. Furthermore, in 17 patients with suspected supra-aortic arteritis, Cyran et al. showed a positive significant strong correlation between mean FDG TBR and DCE-MRI extraction fraction. The discrepancy in correlations may relate to the magnitude of plaque inflammatory activity at the time of noninvasive imaging. Beyond prior studies, the work presented here not only correlated the noninvasive imaging markers with each other but also validated histopathologic markers of inflammation and microvascularization to provide insight into the biological state of the tissue, not just the presence of lesions or cells.

Ample evidence links plaque microvessels to plaques’ propensity to provoke thrombotic complications. Yet, under certain conditions, microvessels may provide a portal for influx of inflammatory cells and lipids. This potential dual role of microvessels in plaque biology may underlie the nonlinear trend observed between rising tertiles of CD31 staining and inflammation by FDG TBR or microvessel permeability by Ki\textsuperscript{trans} (Figure 3).

Limitations of this study include the physiological interpretation of the PET and DCE-MRI imaging parameters themselves. FDG PET furnishes a sensitive and reproducible technique for quantifying uptake of the glucose analog, \(^{18}\)FDG, by metabolically active cells such as proinflammatory macrophages. Yet, the strict correlation of the FDG signal with inflammation remains incompletely defined. We have shown in human monocyte-derived macrophages that hypoxia, but not inflammatory activation, increases glucose uptake. The relationship between glucose utilization by cells and FDG uptake and accumulation depends on the intracellular phosphorylation activity of hexokinases, as well as the specific radioactivity of the glucose analog in the extracellular milieu. Regions of plaques rich in microvessels may have facilitated local delivery of the tracer, increasing its specific radioactivity in the precursor pool for glucose transport. The regional elevation in the marker of permeability Ki\textsuperscript{trans} supports the concept of increased delivery of the isotopic tracer to plaques. Thus, increased FDG signal could reflect regional enrichment of the labeled glucose analog and not an absolute increase in glucose transport. The design of this study did not permit determination of which factors account exactly for FDG uptake. In addition, the Ki\textsuperscript{trans} parameter in DCE-MRI can have different interpretations depending on the assumptions made for kinetic modeling, such as the balance between capillary permeability and perfusion at the location of interest. Furthermore, inherent differences in spatial resolution between imaging modalities preclude perfect correlations between the colocalized measurements and likely contribute to scatter in these data. In the future, hybrid PET/MRI technologies may facilitate ever closer comparisons across multiple imaging modalities.

Despite these limitations and unsettled areas, this study firmly links neovascularization to ongoing inflammation (as assessed by molecular markers in situ) in human atheroma, independent of plaque anatomy, providing in vivo validation in humans of mechanisms hypothesized based on ex vivo observations. Beyond the mechanistic insight, determining whether the application of these molecular and dynamic imaging techniques can improve cardiovascular risk stratification or direct therapy in an effective manner will require prospective studies evaluating their impact on clinical outcomes.

Sources of Funding
This research was supported by the Donald W. Reynolds Foundation.

Disclosures
None.

References


**CLINICAL PERSPECTIVE**

In human carotid disease, current diagnostic and therapeutic guidelines emphasize symptoms and luminal stenosis severity in patient selection for invasive revascularization, yet these indices may lack sensitivity and specificity for predicting optimally the risk of future atherothrombotic events. Molecular and pharmacokinetic imaging techniques, such as 2-deoxy-2-[18F]fluorogluose positron emission tomography/computed tomography and dynamic contrast–enhanced magnetic resonance imaging, allow measurement of metabolic activity and neovascularization, respectively. Although studies have shown the feasibility of imaging plaques with 2-deoxy-2-[18F]fluogluose positron emission tomography and dynamic contrast–enhanced magnetic resonance imaging, and some have attempted limited correlation between histological staining and positron emission tomography or magnetic resonance imaging, inconsistent results have emerged, perhaps underscoring that the mere presence of macrophages or microvessels may not reflect the prevailing inflammatory activity. Indeed, mononuclear phagocytes exhibit considerable functional diversity, such that simply enumerating these cells discloses little about their state of inflammatory activation. This study demonstrated that (1) 2-deoxy-2-[18F]fluorogluose uptake by positron emission tomography and (2) microvascular permeability and perfusion by dynamic contrast–enhanced magnetic resonance imaging correlated with histological markers of macrophage content, active inflammation, and plaque microvascularization, and (3) 2-deoxy-2-[18F]fluorogluose uptake correlated with microvascular permeability, independently of anatomic stenosis severity. As such, this study firmly links neovascularization to ongoing inflammation in human atheroma, independent of luminal narrowing, providing in vivo validation in humans of mechanisms hypothesized based on ex vivo observations. Our results reaffirm the need for prospective testing of whether these molecular and dynamic imaging techniques can improve cardiovascular risk stratification and direct therapy to better clinical outcomes.
Increased Microvascularization and Vessel Permeability Associate With Active Inflammation in Human Atheromata

Viviany R. Taqueti, Marcelo F. Di Carli, Michael Jerosch-Herold, Galina K. Sukhova, Venkatesh L. Murthy, Eduardo J. Folco, Raymond Y. Kwong, C. Keith Ozaki, Michael Belkin, Matthias Nahrendorf, Ralph Weissleder and Peter Libby

*Circ Cardiovasc Imaging*, 2014;7:920-929; originally published online August 28, 2014; doi: 10.1161/CIRCIMAGING.114.002113

Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2014 American Heart Association, Inc. All rights reserved.

Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circimaging.ahajournals.org/content/7/6/920

Data Supplement (unedited) at:

http://circimaging.ahajournals.org/content/suppl/2014/08/28/CIRCIMAGING.114.002113.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Imaging can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:

http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Imaging is online at:

http://circimaging.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Increased Microvascularization and Vessel Permeability Associate with Active Inflammation in Human Atheromata

Viviany R. Taqueti*, MD, MPH, Marcelo F. Di Carli*, MD, Michael Jerosch-Herold, PhD+, Galina K. Sukhova*, PhD, Venkatesh L. Murthy**, MD, PhD, Eduardo J. Folco*, PhD, Raymond Y. Kwong**, MD, MPH, C. Keith Ozaki†, MD, Michael Belkin*, MD, Matthias Nahrendorf, MD, PhD, Ralph Weissleder‡, MD, PhD, Peter Libby*, MD

*From the Heart and Vascular Institute, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.
†From the Noninvasive Cardiovascular Imaging Program, Nuclear Medicine and Molecular Imaging Division, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.
‡From the Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.
**From the Divisions of Nuclear Medicine, Cardiothoracic Imaging, and Cardiovascular Medicine, Departments of Medicine and Radiology, University of Michigan, Ann Arbor, MI, USA.

Correspondence to:
Dr. Peter Libby
Heart and Vascular Center
Brigham and Women’s Hospital
Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115
tel 617-732-0806
fax 617-264-5111
plibby@rics.bwh.harvard.edu
Supplemental Table. Summary Statistics for SUV\textsubscript{BW}, TBR and K\textsubscript{trans}

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean (se)</th>
<th>Mean (se)</th>
<th>Mean (se)</th>
<th>Mean (se)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>SUV\textsubscript{BWmax}</td>
<td>SUV\textsubscript{BWmean}</td>
<td>TBR\textsubscript{max}</td>
</tr>
<tr>
<td>Plaque</td>
<td>53</td>
<td>2.23 (0.07)</td>
<td>1.81 (0.06)</td>
<td>1.76 (0.04)</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>1.59 (0.05)</td>
<td>1.31 (0.05)</td>
<td>1.24 (0.03)</td>
</tr>
<tr>
<td>Blood Pool</td>
<td>53</td>
<td>1.30 (0.03)</td>
<td>1.08 (0.03)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Means with standard errors (se) are shown. SUV\textsubscript{BWmax} and SUV\textsubscript{BWmean} denote maximum and mean standardized uptake values (standardized to body weight, and averaged from 3 measurements recorded along contiguous imaging slices for each region per patient). TBR\textsubscript{max} and TBR\textsubscript{mean} denote maximum and mean target-to-background ratios for 2-Deoxy-2-[\textsuperscript{18}F]fluoroglucose, respectively.