Bilateral Symmetry of Human Carotid Artery Atherosclerosis

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Background and Purpose—Atherosclerosis is a principal cause of stroke and myocardial infarction. The carotid arteries provide a site at which progression of atherosclerosis can be monitored reproducibly and noninvasively. This study was conducted to determine the similarity of atherosclerotic plaques in the left and right carotid arteries. This question was explored with the use of perfusion-fixed cadaveric carotid arteries and 2 noninvasive clinical imaging techniques, MRI and electron-beam CT.

Methods—Fifty pairs of carotid arteries from cadaveric donors (aged 48 to 98 years) were imaged with MRI and electron-beam CT. Thirty-eight of the pairs met the criteria for rigorous analysis. Carotid artery wall volumes were measured from the MRI images, and calcification scores were computed from the electron-beam CT images.

Results—Total wall volumes of the left (972.5 ± 241.6 mm³) and right (1016.3 ± 275.0 mm³) carotid arteries were moderately correlated (concordance correlation coefficient $r_c = 0.71$). Calcification scores were highly correlated, with $r_c = 0.95$ for the Agatston scores and $r_c = 0.94$ for the calcium volume scores.

Conclusions—Total wall volume and plaque calcification in the left and right human carotid arteries are substantially similar. These results suggest that atherosclerosis of the human carotid arteries is generally a bilaterally symmetrical disease. This evidence of symmetry suggests that diagnostic information about atherosclerotic plaque in one carotid artery can be used to infer information about the composition and volume of atherosclerotic plaque in the contralateral artery. (Stroke. 2002;33:2575-2580.)

Key Words: atherosclerosis ■ calcium ■ carotid arteries ■ magnetic resonance imaging

Atherosclerosis of the carotid arteries is a major cause of stroke and transient ischemic attack. Plaques typically form in the common carotid and extend distally into the internal carotid artery. An important question is whether atherosclerotic plaques present in the left and right carotid arteries of the same person exhibit similar composition and volume. This question is particularly important in longitudinal clinical trials that begin with a unilateral carotid endarterectomy. If carotid artery atherosclerosis is a symmetrical disease, then lesions observed in an operative specimen ex vivo should resemble and reflect lesions present in the contralateral vessel in vivo. Determining the volumes of plaque components and comparing them within intrasubject pairs of arteries would be a rigorous method of quantitatively assessing atherosclerotic plaque symmetry in the carotid arteries. Currently it is technically challenging to quantify all plaque components. However, one plaque component that can be unequivocally identified and accurately quantified is calcification. Arterial volumes, including lumen volume, wall volume, and total volume, can also be quantified. It is possible to estimate plaque volume and normal wall volume from the wall volume measurements.

Since different arteries within the same individual are exposed to the same environment, atherosclerotic plaque development might progress at the same rate. However, differences in arterial geometry and flow dynamics could result in different plaque burdens. This question can be examined in paired arterial beds such as the carotid arteries. Solberg et al measured the percentage of intimal surface involved with raised atherosclerotic lesion in arterial autopsy samples and found strong correlations within paired arteries, particularly the carotids. Howard et al compared carotid intimal-medial thickness measured using B-mode ultrasound in the left and right carotid arteries in the Atherosclerosis Risk in Communities study. The correlations were 0.49 for the common carotid (9386 paired measurements), 0.34 for the bifurcation (5748 paired measurements), and 0.38 for the internal carotid (3202 paired measurements). Vink et al examined paired human femoral artery autopsy specimens and found a significant correlation of plaque volume between left and right.

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femoral arteries ($r^2=0.5$). The presence of a large lipid-rich core was also correlated within left and right femoral arteries ($\kappa=0.60$). The presence of inflammation within the cap and shoulders of the plaque was not correlated within the paired arteries ($\kappa=0.067$). To determine the symmetry of atherosclerosis in human carotid arteries, we acquired 50 pairs of perfusion-fixed cadaveric human carotid arteries. Perfusion-fixed cadaveric specimens maintain in vivo geometry after excision and are stable over time. Carotid artery volumes can be accurately quantified with the use of high-resolution MRI techniques.6,7

The amount of calcification present within the plaque can be accurately measured with the use of electron-beam computed tomography (EBCT).8–10

In this study we report the degree of symmetry present in 38 pairs of cadaveric human carotid arteries. This study tests the hypothesis that in intrasubject left and right carotid arteries, the volumes and calcification levels of atherosclerotic plaques are similar as determined by MRI and EBCT. Artery volumes, including total artery, lumen, and total wall volume, were measured by high-resolution MRI. Total wall volume was subdivided into normal wall and plaque volume with the use of an automated estimation algorithm. Plaque calcification was quantified with the use of EBCT.

Methods

Sample Acquisition

Fifty paired human carotid trees were harvested from perfusion-fixed cadavers willed to the Baylor College of Medicine anatomy laboratory. Cadavers were embalmed with 10% formalin and stored at room temperature for up to 2 years before the tissues were harvested. Trained personnel excised the left and right carotid trees, maintaining intact as much of the artery as possible, including the intima, media, and adventitia. Average specimen length was approximately 9 cm, with 4 cm distal and 5 cm proximal to the bifurcation. Twelve sample pairs were rejected as unsuitable for analysis. Ten of the sample pairs included a specimen that did not contain sufficient length in either the internal, external, or common carotid branch. Two pairs, although of sufficient length, had been accidentally cut through an atherosclerotic plaque. Thirty-eight of the carotid pairs were suitable for rigorous comparison. Demographic information was available for 37 of the 38 sample pairs. Mean±SD age of the patients at death was 79±12 years (range, 48 to 98 years). Cardiovascular disease (stroke, coronary artery disease, myocardial infarction, congestive heart failure) was listed as a cause of death for 18 of the 38 donors.

Magnetic Resonance Imaging

Carotid artery pairs were imaged on a GE Horizon 1.5-T clinical MRI system with the use of Ultralmage-Pathway phased-array coils specifically designed for in vivo carotid artery imaging. Two carotid pairs were positioned in a specifically designed and fabricated holder that accommodated four 50-mL plastic culture tubes, each containing 1 artery (Figure 1). This holder was filled with room temperature water and positioned within the MRI scanner with the long axis of the arteries perpendicular to the $z$ axis of the magnet. The MRI parameters are listed in Table 1. Total imaging time was approximately 30 minutes per 2 carotid pairs.

**EBCT Imaging**

EBCT was performed with the use of an Imatron C150 clinical system. The imaging parameters used were a 40-cm field of view, slice thickness of 3 mm, and a 512×512 matrix. Twelve pairs of carotid arteries were imaged simultaneously. Both Agatston scores and volumetric scores for calcification were computed for each sample with the use of AccuImage software. All of the carotid specimens were imaged twice, with an interscan interval of 141 days.

**Volume Measurements From MRI**

Carotid artery volumes were measured on the MR images with the use of a semiautomatic active contour algorithm11 to define the outer boundary of the artery, which gave the total artery area, and the boundary of the lumen, which gave the lumen area. The measured area was multiplied by the slice thickness to calculate the slice volume. In some cases postmortem clot was present within the lumen of the carotid specimen. The boundary between the clot and the lumen wall was clearly demarcated. The outer boundary of the artery was visible as a continuous dark line on the T2-weighted images. The generalized gradient vector force field12 was used as the external force for the active contour. An anisotropic diffusion filter was used to reduce the noise of the MR images.13,14 To perform a measurement, the operator manually defined an initial contour near the desired boundary, and the contour algorithm deformed the contour to fit the boundary. A single operator performed all of the measurements. Each sample was measured 3 times on separate days. Total wall volume was defined as the total artery volume minus the lumen volume.

Plaque volume estimation was based on subtracting the estimated normal wall volume from the total wall volume. The normal wall volume for each slice was estimated with the use of the 10th percentile of wall thickness distribution for that slice. The 10th percentile is less affected by variation in the contours caused by

<table>
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<th>TABLE 1. MRI Acquisition Parameters</th>
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<tr>
<td><strong>Contrast</strong></td>
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<tr>
<td>Repetition time, ms</td>
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<td>Echo time, ms</td>
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<td>Field of view, cm</td>
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<tr>
<td>Matrix size</td>
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<tr>
<td>No. of excitations</td>
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<td>Slice thickness, mm</td>
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image noise than the true minimum. The estimated normal wall thickness from each MRI slice was averaged for the internal, external, and common carotid artery branches to determine an estimate of the normal wall thickness for each branch. Shifting the contour enclosing the entire artery toward the lumen contour by the estimated normal wall thickness generated a contour for the inside of the normal wall. Plaque volume was defined as total wall volume minus normal wall volume. Percent stenosis was defined as the estimated plaque volume divided by the potential lumen volume, which was defined as the observed lumen volume plus the estimated plaque volume.

For comparison purposes, slices in the left and right carotid arteries were coregistered by their distances from the bifurcation of the common carotid into the internal and external carotids. The location of the bifurcation was defined as the most proximal image slice in which the lumens of the internal and external carotid branches were visible as 2 completely separate orifices. Aggregate volumes were computed for 9 contiguous slices bounding the bifurcation (ie, 4 slices above and 5 below). The measurement algorithm is depicted in Figure 2.

Statistical Analysis
Descriptive statistics are presented as mean±SD. Variation of the EBCT data from 2 separate imaging sessions was computed as the absolute difference between a pair of measurements divided by the mean of the measurements. The coefficient of variation was used to compute the reproducibility of the 3 replicate MRI volume measurements made on a single set of image data. Average volumes over the 38 sample pairs were computed for each 3-mm slice registered by distance from the bifurcation. Symmetry was evaluated by comparing aggregate artery volumes and calcification scores between the 2 members of each artery pair. Lin’s concordance correlation coefficients \( r_c \) were used to quantify the agreement between left and right carotid artery aggregate volumes and calcification scores within the carotid pairs. The Spearman correlation coefficient \( r_s \) was used to determine the relationship between calcification scores from EBCT and volume measurements from MRI.

Results

Reproducibility of Measurements
The reproducibility of the EBCT calcification scores \((n=76)\), with median variation of 6.2% for the Agatston score and 4.8% for the volumetric score, is comparable to in vivo imaging of coronary arteries, with reported median variation of 7.8% for the Agatston score and 5.7% for the volumetric score.\(^{16}\) The time interval between EBCT imaging sessions was 141 days. The median coefficient of variation for the measured aggregate volumes from MRI \((n=76)\) was 0.9% for the lumen volume, 1.0% for the total artery volume, and 1.9% for the total wall volume. The median coefficient of variation for the estimated aggregate volume \((n=76)\) was 0.8% for the normal wall volume, 4.7% for the plaque volume, and 3.5% for the percent stenosis.

Slice Volume Profiles
The composite slice volume profiles are presented in Figure 3A to 3E. Volumes within the common carotid branch are largest near the bifurcation and decrease in slices farther from the bifurcation, becoming constant in the slices farthest from the bifurcation. A similar pattern is observed in the internal and external carotid branches. The same pattern is present when displayed as percent stenosis, with the greatest amount of stenosis near the bifurcation (Figure 3F). Average slice volumes of left (dotted lines) and right (solid lines) carotids are similar for all of the measured and estimated volumes at all offsets. At most offsets the average slice volumes of the left carotid are slightly but not substantially smaller than those of the right carotid.
Aggregate Volumes and Calcification Scores

The aggregate volumes were computed for 9 contiguous slices bounding the bifurcation of each of the samples in the 38 carotid pairs. The descriptive statistics for the aggregate volumes are presented in Table 2. There was no significant difference between any of the mean volumes of the left and right carotids. The Agatston score and the volumetric score were highly correlated, with a Spearman correlation coefficient of 0.997 for the left samples (n=38) and 0.998 for the right samples (n=38).

The concordance correlation coefficients comparing left and right carotid aggregate volumes from MRI and calcification scores from EBCT are presented in Figure 4. The Agatston scores and calcium volume scores of the left and right carotid arteries were highly correlated, with \( r_c = 0.95 \) and \( r_c = 0.94 \), respectively. Total wall volume, which is a surrogate marker for atherosclerotic plaque volume, had the highest concordance correlation coefficient of the MRI volumes, with \( r_c = 0.71 \). The estimated plaque volume had a lower concordance correlation coefficient of \( r_c = 0.58 \).

Correlation of Carotid Volumes and Calcification

Volume measurements from the MRI images were compared with Agatston calcification scores from the EBCT images. Lumen volume was not significantly correlated with the amount of calcification: \( r_s = -0.01 \) for the left and \( r_s = -0.30 \) for the right. Total wall volume (left \( r_s = 0.59 \), right \( r_s = 0.36 \)) and plaque volume (left \( r_s = 0.53 \), right \( r_s = 0.34 \)) were moderately correlated on the left but not on the right. Percent stenosis (left \( r_s = 0.53 \), right \( r_s = 0.50 \)) was moderately correlated with the Agatston score on both left and right sides.

Discussion

This study tested the hypothesis that the volume of atherosclerotic plaque and calcification is similar in left and right carotid arteries. The aggregate volumes were computed for 9 contiguous slices bounding the bifurcation of each of the samples in the 38 carotid pairs. The descriptive statistics for the aggregate volumes are presented in Table 2. There was no significant difference between any of the mean volumes of the left and right carotids. The Agatston score and the volumetric score were highly correlated, with a Spearman correlation coefficient of 0.997 for the left samples (n=38) and 0.998 for the right samples (n=38).

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Figure 3. Average slice volume profiles from MRI. Plots of the average slice volume (A to E) and percent stenosis (F) of the 38 specimens registered by distance from the bifurcation are shown. Only points with at least 10 measurements in both the left and right carotid arteries are plotted. The dashed lines are the averages for the left carotid artery. The solid lines are the averages for the right carotid artery. C indicates slice offsets in common carotid artery; B, slice identified as bifurcation; and I/E, slice offsets in internal and external carotid arteries. Volumes from the common carotid are indicated by a square; internal carotid by a circle; and external carotid by a diamond.

Table 2. Aggregate Volume and Calcification Score Descriptive Statistics

<table>
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<tr>
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<th>Range</th>
<th>Left Carotid</th>
<th>Right Carotid</th>
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<tr>
<td>Lumen</td>
<td>419–1588</td>
<td>972±241</td>
<td>1016±275</td>
</tr>
<tr>
<td>Total artery</td>
<td>1640–3947</td>
<td>2455±456</td>
<td>2557±529</td>
</tr>
<tr>
<td>Total wall</td>
<td>652–2248</td>
<td>1482±343</td>
<td>1541±365</td>
</tr>
<tr>
<td>Normal wall</td>
<td>496–1662</td>
<td>981±219</td>
<td>1022±219</td>
</tr>
<tr>
<td>Plaque</td>
<td>156–989</td>
<td>501±159</td>
<td>518±184</td>
</tr>
<tr>
<td>Percent stenosis</td>
<td>13.7–62.4</td>
<td>34.0±9.2</td>
<td>33.9±8.2</td>
</tr>
<tr>
<td>Agatston score</td>
<td>0–1956</td>
<td>417±512</td>
<td>411±523</td>
</tr>
<tr>
<td>Calcium volume score</td>
<td>0–1505</td>
<td>334±405</td>
<td>330±416</td>
</tr>
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Values are ranges and mean±SD, expressed in cubic millimeters.
The moderate level of symmetry of wall and plaque volumes between left and right carotid arteries suggests that the development of atherosclerosis is partially controlled by systemic factors such as plasma cholesterol levels. The variation between left and right volumes suggests that local factors are also important. Vink et al. demonstrated that there is very little concordance in plaque inflammatory state between the left and right femoral arteries. Other local factors that may play a role in the asymmetry of atherosclerotic plaque burden include variations in vessel anatomy, particularly bifurcation anatomy, and differences in wall shear stress.

The perfusion-fixed cadaveric carotid artery specimens used in this study provided a stable, convenient ex vivo model for studying human carotid atherosclerosis. Imaging measurements on the specimens were inherently more precise and accurate compared with measurements on patients, since there were no motion artifacts and there was no practical limit on imaging time. Since the specimens were removed en bloc, all 3 layers of the arterial wall and some periadventitial soft tissue were present. In carotid endarterectomy specimens, however, only the intima and a thin layer of the innermost media are usually present.

MRI has been used in single-center clinical trials to track changes in carotid and aortic atherosclerotic plaque burden. A potential use for the cadaveric tissues is as a reference standard for validating the reproducibility of MRI across multiple centers. If images of the cadaveric specimens could be acquired and processed similarly at different centers with the use of a similar sample holder, the resulting images can be compared directly to determine the reproducibility of the arterial volume measurements and of the image intensities, which indicate different tissue types.

MR and EBCT imaging measurements were performed with clinical scanners. MRI was conducted with the use of the same phased-array coils that we have used for in vivo imaging of human carotids. MRI scan time for a single session, during which 2 carotid pairs could be imaged, was approximately 30 minutes. EBCT imaging was much faster, with a scan time per session of approximately 2 to 3 minutes; 12 carotid pairs were imaged per session. Together, MRI and EBCT provided complementary information that neither imaging modality could have provided separately.

Using a semiautomated active contour algorithm to locate the lumen and artery wall boundaries greatly decreased the time required to measure all of the arterial volumes. The plaque estimation algorithm is based on 3 assumptions: (1) the normal wall thickness is the minimum measured wall thickness; (2) the normal wall thickness is constant around the circumference of the artery; and (3) the normal wall thickness is constant within the individual branches of the carotid artery. Using these assumptions and the plaque estimation algorithm, we were able to obtain a reproducible estimate of the volume of plaque present within the carotid artery tissues.

EBCT provided an accurate method for quantifying calcification, an important component of carotid atherosclerotic plaque. Both Agatston and calcium volume scores were calculated. The reproducibility of the calcium volume score was slightly better than that of the Agatston score. The reproducibility of the EBCT calcium scores from this study compared favorably with the variability of calcium scores measured with the use of in vivo imaging of coronary arteries.

The average slice volume profiles indicate that the average arterial slice volumes are similar in left and right carotids in each individual. The arterial volumes tend to be greatest in slices proximal to the bifurcation and to decrease in slices farther from the bifurcation. All of the aggregate arterial volumes measured by MRI were moderately correlated between left and right carotid arteries. Since aggregate volumes were compared, it is possible that the plaque is distributed differently in the 2 arteries. The same degree of symmetry may not be observed in studies in which angiography is used because that technique is limited to observing only the lumen of the artery. The samples in this study were not acquired from symptomatic patients being evaluated for carotid endarterectomy. That population, which usually has large occlusive plaques, may or may not exhibit the same degree of symmetry.

Calcification was slightly negatively correlated with lumen volume and was moderately correlated with plaque volume and percent stenosis. This implies that increased calcification results in decreased lumen volume and is associated with larger lesions. The relatively low correlation values are partially caused by some samples containing large volumes of uncalcified plaque. Thus, while large amounts of detectable calcification suggest that plaque is present, the absence of calcification does not indicate that plaque is not present. Recent studies have demonstrated that calcification of atherosclerotic plaque is an active, cell-mediated process, with many of the same proteins involved in regulating bone formation present within calcified atherosclerotic plaques. The high correlation between calcification volume in the left and right carotid arteries suggests that calcium deposition may be regulated by systemic factors.

This study compared only arterial volumes and a single plaque component, calcification. A technique that holds high promise for identification and quantification of other plaque components is multispectral MRI. Using concepts adapted from earth satellite imagery, this technique employs multiple contrast images to identify plaque components. Yuan et al. have demonstrated that multispectral MRI can qualitatively identify lipid-rich necrotic cores within in vivo images of
human carotids. The thickness of the fibrous cap overlying the necrotic core can be determined with the use of MRI.28

Another question that this study raises is how atherosclerotic plaque volume in the carotid arteries compares with plaque volume within other arteries. Vink et al3 have shown that atherosclerotic plaque burden is moderately symmetrical in the femoral arteries. The described imaging techniques could be used to quantify arterial volumes in other paired arteries either in vivo or ex vivo if appropriate specimens could be obtained. Thus, lesion symmetry in paired arterial beds, such as the vertebral, renal, popliteal, and femoral arteries, could also be quantified.

Acknowledgments

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