Differences between carotid wall morphological phenotypes measured by ultrasound in one, two and three dimensions

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Abstract

Ultrasound measurements are both surrogate markers and risk factors for atherosclerosis end points. Carotid intima-media thickness (IMT) is most commonly used, but ultrasound can also define structures in higher spatial dimensions, such as total plaque area (TPA) and total plaque volume (TPV). Because there are minimal data regarding the relationship between IMT, TPA and TPV, we measured these variables in 272 Oji-Cree subjects. We found pairwise correlations for IMT:TPA, IMT:TPV and TPA:TPV of 0.507, 0.588 and 0.846, respectively (transformed variables, all $P<0.0001$). In a subset of 168 subjects with complete cardiovascular risk factor data, we performed multivariate regression analysis to identify sources of variation for IMT, TPA and TPV. We found that the ultrasound traits showed different correlations with individual cardiovascular risk factors. In particular, IMT was significantly associated with hypertension, TPA with smoking and plasma cholesterol, and TPV with diabetes. Therefore, these ultrasound measures of carotid artery morphology, while somewhat correlated, likely represent distinctive quantitative traits with different biological determinants, as underscored by different risk factor associations in the multivariate regression analysis. Because the measurements have different implications and determinants, investigators might need to be selective about the particular measurements they choose for specific applications.

Keywords: Atherosclerosis; Carotid arteries; Imaging; Plaque; Ultrasonics

1. Introduction

Atherosclerosis is a complex multi-stage, multi-factorial disease process \cite{1,2} that connotes many phenotypes ranging from clinical events to measurements taken from images acquired non-invasively, such as by ultrasound (US). Among carotid US determinations, intima-media thickness (IMT) has been extensively studied both as a surrogate marker of atherosclerosis and a predictor of clinical events \cite{3-12}. IMT is a linear variable acquired using B-mode ultrasonography, capturing images of a standardized portion of the carotid arterial wall, measuring combined thickness of intima and media at specified intervals and then determining the mean of these \cite{5-12}. Some groups have used 2-dimensional (2D) US assessment of carotid plaque area \cite{13-19}, in our studies, a reader manually quantified cross-sectional areas of all carotid plaques visualized in a specified region and their sum was reported as total carotid plaque area (TPA) \cite{14-19}. Even more recently, attempts have been made to quantify carotid...
plaque volume with US [20–24]; in this approach, a reader manually traces plaque borders within cross-sectional planes at specified intervals. Computer software reconstitutes a 3-dimensional (3D) plaque image using spatial co-ordinates, and volumes are summed and reported as total carotid plaque volume (TPV) [20–25].

Although IMT, TPA and TPV each represent a morphological or anatomical attribute of the carotid arterial wall, each might actually measure a different aspect of the disease process. For instance, IMT may reflect wall hyperplasia or hypertrophy related to hypertension, whereas the assessments of plaque size, either TPA or TPV necessarily reflect a more advanced stage of atherosclerosis, which may be related to foam cell formation or thrombosis. In order to understand whether there is a relationship between IMT, TPA and TPV, we concurrently measured these attributes in the same group of individuals. We evaluated their correlation with each other and their association with traditional cardiovascular risk factors. We found moderate correlations between them. Each had distinctive associations with cardiovascular risk factors.

2. Methods
2.1. Study sample
We studied residents of an aboriginal community in central Canada. Residents are Oji-Cree and demographic, clinical and biochemical attributes have been described previously [26–31]. Two hundred seventy-two adult community members had US assessment of the carotid arteries. Complete cardiovascular risk factor data were available in a subset of 168 subjects. This assessment included: (1) determination of the presence or absence of medically diagnosed diabetes using either previous medical diagnosis or oral glucose challenge, as described in [26,27]; (2) plasma lipoproteins determination, as described in [28,29]; (3) determination of the presence or absence of current cigarette smoking, as described in [26–31] and (4) determination of the presence or absence of medically diagnosed and/or treated hypertension, as described in [30,31]. All subjects provided written informed consent and the study was approved by the aboriginal band council and the Mt. Sinai Hospital Research Ethics Board.

2.2. General ultrasound logistics
Ultrasound (US) images were obtained using an HDI-5000 US machine and an L12-5 transducer (both from Advanced Technology Laboratories, Bothel, WA) that had been flown to the community and housed within the project research facility. A single-certified operator used the same instrument over a 4-week period to obtain carotid US images suitable for determination of IMT, TPA and TPV from each of 272 subjects.

2.3. IMT measurement
A single observer, blinded to the subjects’ demographic data and cardiovascular risk, measured combined thickness of intima and media of the far wall of both common carotid arteries, assessed IMT. Images were recorded from an anterolateral longitudinal view. These views were played back using an image processing board and a specialized recorder with digital memory permitted the digitization of a full video frame in still mode. The still images were analyzed using computerized edge-detection software (Prowin™) [32]. Using a step-wise algorithm, conditional sets of “edges” (consisting of lumen-intima and media-adventitia echoes) were located within the image and then tested them for “edge strength”. Weak edge points were deleted, thus minimizing the identification of spurious edge points due to image noise. Once all acceptable edge points were identified, boundary gaps were filled by linear interpolation. The distance between lumen-intima and media-adventitia boundaries was then measured to calculate IMT. Mean IMT was computed from 80 to 120 measurements over a 10 mm span ending 5 mm proximal to the transition between the common carotid and bulb regions. Intra- and inter-operator coefficients of variation of 3.0 and 3.1%, respectively, and intra- and inter-operator intraclass correlations were both 0.97.

2.4. TPA measurement
TPA was measured as described previously [14–19]. Briefly, plaque was defined as a local intimal thickening >1 mm. Measurements were made in magnified longitudinal views of each plaque in common, internal and external carotid artery bilaterally. The measurement plane was determined by scanning to find the largest extent of plaque. The image was then frozen, magnified, and the plaque was measured by tracing around the perimeter with a cursor on the screen. The microprocessor in the scanner then displayed the cross-sectional area of the plaque. The process was repeated until all plaques on both sides were measured. TPA was the sum of cross-sectional areas of all plaques between the clavicle and angle of the jaw. Blinded intra- and inter-observer intraclass correlations were 0.94 (N = 50) and 0.85 (N = 50), respectively.

2.5. TPV measurement
Data from the 2D US TPA studies were used to localize plaques. The resulting 2D US images that were parallel to each other within a known regular spatial interval and constant transducer angle were immediately reconstructed into a 3D volume to verify scan quality [24,25]. 3D US images were acquired with a mechanical linear scanning system and analyzed with 3D visualization software (Life Imaging Systems Inc., London, Ont.). Each 3D image was displayed using multiplanar texture mapping, allowing plaques to be viewed in various orientations. Plaque volumes were measured using
manual planimetry: each 3D image was ‘sliced’ transversely at an inter-slice distance of 1 mm, moving from one plaque edge to the other. Plaque boundaries were traced using a mouse driven cross-hair cursor. Slice areas were summed and multiplied by inter-slice distance to calculate plaque volume. TPV was the sum of all plaque volumes between the clavicle and angle of the jaw for both carotids. Intra- and inter-observer reliabilities were 0.94 (N = 40) and 0.93 (N = 40), respectively [25].

### 2.6. Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical comparisons. For pairwise correlations between variables, we used data from all 272 subjects. Transformations to normalize variable distributions and tertiles for each analyte were also created. Pearson correlation analyses between variables were performed with and without normalization, and for tertiles of each variable. A subset of 168 subjects who had US determinations also had full cardiovascular risk factor data. Multivariate linear regression analysis was used to determine sources of variation for transformed carotid US measures, using selected continuous and discrete risk factor traits as covariates.

### 3. Results

#### 3.1. Baseline attributes of study subjects

The age (mean ± S.D.) of the total 272 study subjects was 44.7 ± 14.6 years, and 40.1% were male. In the entire sample, IMT, TPA and TPV were 0.77 ± 0.16 mm (range: 0.49–1.42 mm), 0.39 ± 0.61 cm² (range: 0–3.92 cm²) and TPV 83.4 ± 166 mm³ (range: 0–1248 mm³), respectively. Demographic attributes of the subset of 168 subjects, who were studied further with multivariate regression analysis, are shown in Table 1.

#### 3.2. Correlations between traits

Inverse transformation of IMT (1/IMT), natural logarithmic transformation of TPA and cube root transformation of TPV produced variables with distributions that did not significantly deviate from normal. Pearson correlation coefficients in every pairwise analysis were highly significant (Table 2, all \(P < 0.0001\)). For the IMT:TPA pair, \(r = 0.724, 0.507\) and 0.647 for correlations of untransformed variables, transformed variables and tertiles, respectively. For the IMT:TPV pair, \(r = 0.677, 0.588\) and 0.615 for correlations of untransformed variables, transformed variables and tertiles, respectively (Table 2). For the TPA:TPV pair, \(r = 0.934, 0.846\) and 0.827 for correlations of untransformed variables, transformed variables and tertiles, respectively. Thus, the variables were significantly correlated, but the correlation coefficients were somewhat smaller for pairwise comparisons of IMT and either TPA or TPV than the correlation coefficient for the TPA and TPV pairwise comparison.

#### 3.3. Multivariate linear regression analysis

Sources of variations for these transformed US variables were determined using multivariate linear regression analysis, with independent variables of age, sex, hypertension history, current smoking history, plasma total cholesterol concentration and diabetes history. The nominal level of significance was set at \(P < 0.05\). Results are shown in Table 3. Age and sex were associated with all three US determinations. However, there were differences in the associations for US measures with the remaining cardiovascular risk factors. For instance, transformed IMT was significantly associated only with hypertension, but not with smoking, cholesterol or

### Table 1

Demographic data for subjects in cardiovascular risk assessment

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>168</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>Quantitative traits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.2 ± 4.9</td>
<td>37.2 ± 5.6</td>
<td>38.8 ± 4.8</td>
</tr>
<tr>
<td>Plasma total cholesterol (mM)</td>
<td>4.77 ± 0.91</td>
<td>4.83 ± 0.97</td>
<td>4.73 ± 0.88</td>
</tr>
<tr>
<td>Plasma triglycerides (mM)</td>
<td>1.76 ± 0.94</td>
<td>1.82 ± 0.98</td>
<td>1.72 ± 0.94</td>
</tr>
<tr>
<td>Plasma HDL, cholesterol (mM)</td>
<td>1.19 ± 0.26</td>
<td>1.13 ± 0.28</td>
<td>1.24 ± 0.24</td>
</tr>
<tr>
<td>Plasma LDL, cholesterol (mM)</td>
<td>2.77 ± 0.73</td>
<td>2.88 ± 0.83</td>
<td>2.68 ± 0.65</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.0 ± 5.99</td>
<td>27.9 ± 5.4</td>
<td>29.8 ± 5.52</td>
</tr>
<tr>
<td>Mean carotid IMT (mm)</td>
<td>0.77 ± 0.18</td>
<td>0.80 ± 0.19</td>
<td>0.76 ± 0.16</td>
</tr>
<tr>
<td>Total carotid plaque area (cm²)</td>
<td>0.43 ± 0.66</td>
<td>0.52 ± 0.82</td>
<td>0.36 ± 0.52</td>
</tr>
<tr>
<td>Total carotid plaque volume (mm³)</td>
<td>99.7 ± 192</td>
<td>121 ± 232</td>
<td>85.4 ± 158</td>
</tr>
<tr>
<td>Discrte traits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>15.2</td>
<td>13.2</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>30.3</td>
<td>36.8</td>
<td>26.0</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>48.2</td>
<td>39.4</td>
<td>60</td>
</tr>
</tbody>
</table>

Abbreviations: mM, millimole/L; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IMT, intima-media thickness.
Table 2: Correlation matrices of carotid arterial US measures

<table>
<thead>
<tr>
<th></th>
<th>IMT</th>
<th>TPA</th>
<th>TPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT</td>
<td>1</td>
<td>0.724**</td>
<td>0.677**</td>
</tr>
<tr>
<td>TPA</td>
<td>0.934**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TPV</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Significant correlation with $P < 0.0001$.

Table 3: Multivariate regression analysis of ultrasound measures and traditional risk factors

<table>
<thead>
<tr>
<th></th>
<th>Beta (inverse)</th>
<th>P</th>
<th>Beta (log)</th>
<th>P</th>
<th>Beta (cubic root)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>0.050 ± 0.026</td>
<td>0.054</td>
<td>0.535 ± 0.143</td>
<td>&lt;0.0001</td>
<td>0.582 ± 0.213</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.042 ± 0.006</td>
<td>&lt;0.0001</td>
<td>0.042 ± 0.006</td>
<td>&lt;0.0001</td>
<td>0.064 ± 0.006</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.169 ± 0.149</td>
<td>NS (0.26)</td>
<td>0.004 ± 0.226</td>
<td>NS (0.99)</td>
<td>0.325 ± 0.299</td>
<td>NS (0.08)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.384 ± 0.221</td>
<td>0.009</td>
<td>0.384 ± 0.221</td>
<td>0.009</td>
<td>0.384 ± 0.221</td>
<td>0.009</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.194 ± 0.076</td>
<td>NS (0.10)</td>
<td>0.012</td>
<td>NS (0.51)</td>
<td>0.132 ± 0.116</td>
<td>NS (0.26)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.100 ± 0.150</td>
<td>NS (0.51)</td>
<td>0.012</td>
<td>NS (0.51)</td>
<td>0.710 ± 0.221</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: IMT, carotid intima-media thickness; TPA, total carotid plaque area; TPV, total carotid plaque volume.

4. Discussion

We studied three related atherosclerosis phenotypes measured simultaneously in the same vascular bed, namely carotid IMT, TPA and TPV, each measured using US imaging. We found that: (1) while these traits were significantly correlated, pairwise comparisons of IMT with either TPA or TPV had lower correlation coefficients than the comparison of TPA with TPV and (2) associations with risk factors differed substantially between IMT, TPA and TPV. While each was significantly associated with age and sex, IMT was significantly associated only with hypertension, TPA only with smoking and plasma cholesterol, and TPV only with diabetes. The findings suggest that the three different US-derived measures of carotid artery morphology, while somewhat correlated, might represent distinct intermediate traits with unique determinants and relationships with risk factors. Thus, the use of any particular US trait as a surrogate marker for "atherosclerosis" might lead to different conclusions regarding the role of specific risk factors in a particular patient sample. Because the measurements could have different implications and determinants, investigators might need to be selective about the particular measurements they choose for specific applications.

While the simple correlations between IMT and plaque measurements were very statistically significant, the r-values between 0.6 and 0.7 indicated correlations that were only moderate. Fig. 1 shows how correlations between US traits may vary between individuals. The top panels contain images of the right carotid artery with arrows at the far wall showing IMT, and shaded regions showing plaque area and volume for an individual with mean IMT of ~1 mm. In this subject, a large plaque is seen on the near wall, which contributes to the large TPA and TPV. The bottom panels contain images of the right carotid with arrows showing typical IMT, and shaded regions showing plaque area and volume for another individual with mean IMT of ~1 mm. However, for this subject, there was only a long, slender plaque overlying the region...
used for IMT determination. The absence of other plaques contributed to low TPA and TPV for this individual. Thus, IMT does not always reflect total carotid disease burden. As a measurement, IMT has the benefit of standardization of acquisition, but the rigorous standards for the anatomical site interrogated to derive this measure may also exclude some important information about atherosclerotic burden in the remainder of the carotid arterial bed. Since TPA and TPV are determined from the same plaques, it follows that the correlation between these traits is stronger. Recently, we employed a similar study design in a different population to show that TPA and the percent carotid stenosis measured by US were only moderately well correlated and had different associations with specific risk factors [33]. We speculated that TPA might have reflected atherosclerotic lesion size more closely than did percent carotid stenosis. In contrast, percent carotid stenosis might have reflected hemodynamic compromise within the arterial lumen, and as a result would show a different relationship with specific risk factors. In the same way, IMT and plaque measurements (TPA and TPV) likely reflect different attributes of atherosclerosis. IMT probably reflects a hypertrophic response of intimal and medial cells to lipid infiltration or hypertension [34]. In contrast, formed plaques probably represent a later stage of atherogenesis related to inflammation, oxidation and/or myocyte proliferation [1,2]. IMT bears a general relationship with total plaque burden estimated by TPA or TPV, but the traits may be markedly different in an individual subject. Of course, we recognize that our study is somewhat limited by several factors, including a small sample size and a unique aboriginal study sample for which the findings not be generalizable. Obviously, studies of these ultrasound measures in other samples and cohorts would be essential. Thus, specific US measures may have different biological determinants and possibly different associations with future clinical events. These differences should be considered in study design and interpretation. Recent coining of the term “phenomics”, meaning integrated multidisciplinary research to understand the complex consequences of genomic variation [35,36], draws attention to the importance of having well-defined and well-characterised atherosclerosis phenotypes, such as those defined by ultrasound. The results indicate that within the same study sample, atherosclerosis determinants differ in their relationships with particular phenotypes, even when these are correlated with each other and determined using similar imaging modalities in the same vascular bed. This
has implications for studies of determinants of “atherosclerosis” that use indirect surrogate markers determined non-invasively.

Different phenotypes of atherosclerosis should not be regarded as equivalent. Future studies of the determinants of atherosclerosis will require a priori distinction between phenotypes and appreciation that they will not necessarily have similar determinants.

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