Segment-Specific Effects of Cardiovascular Risk Factors on Carotid Artery Intima-Medial Thickness in Women at Midlife

Laura L. Schott, Rachel P. Wildman, Sarah Brockwell, Laurey R. Simkin-Silverman, Lewis H. Kuller, Kim Sutton-Tyrrell

Objective—We investigated associations between segment-specific carotid intima-medial thickness (IMT) and cardiovascular risk factors collected before menopause for insight into mechanisms of atherosclerosis development.

Methods and Results—Participants were 453 healthy women (aged 46 to 58 years) enrolled in a dietary and physical activity randomized clinical trial. Ultrasound scan measures were taken ≈2.7 years after baseline in the common carotid artery (CCA), bifurcation (bulb), and internal carotid artery (ICA) segments. When scanned, 84% remained premenopausal. In linear regression models adjusted for age, menopausal status, and intervention group, measures independently (P<0.05) and positively associated were as follows: baseline weight (β=0.007 per 5 kg), systolic blood pressure (SBP; β=0.008 per 10 mm Hg), and age (β=0.02 per 5 years) with CCA IMT; smoking (β=0.08), weight (β=0.009), and SBP (β=0.02) with bulb IMT; and apoprotein B (β=0.01 per 0.1 g/L) with ICA IMT. Differential effects in a repeated measures model with all 3 IMT locations showed these risk factors to have segment-specific positive associations. The effect of weight was strongest in the CCA, smoking and SBP were specific to the bulb, and apoprotein B was strongest in the ICA segment.


Key Words: atherosclerosis ■ women ■ carotid artery ■ ultrasound ■ cardiovascular risk factors

Carotid intima-medial thickness (IMT) is considered a marker of early atherosclerosis, it predicts future risk of cardiovascular disease, and it has been found to be high in individuals with coronary heart disease and myocardial infarction (IMT≥0.73 mm and ≥1 mm, respectively). Among women of all ages, cardiovascular risk factors, such as high blood pressure, body mass index (BMI), and cholesterol, are associated with average and maximum carotid IMT. However, associations with specific locations of thickening within the carotid artery are not as definitive. Considering risk factors in conjunction with specific segments of IMT may provide better insight into the mechanism of atherosclerotic development. It is likely that differences in hemodynamics, cellular processes, and the risk factors themselves affect the development and progression of disease. Additionally, each site may have its own predisposing factors, and biomechanical and biochemical pathways may be acting in combination to influence atherosclerosis.

The purpose of this report is to evaluate atherosclerotic risk factors and IMT by carotid artery segment in a sample of 453 healthy women aged 46 to 58 years. In addition to using standard linear regression models, we use a repeated measures model to provide evidence of segment-specific pathways for development of IMT. Investigating risk factor associations with all 3 segments in the same model has not been done in previous studies.

Methods

Participants
Participants were recruited from the 535 women (92% white) who participated in the Women’s Healthy Lifestyle Project, an intervention trial designed to evaluate the efficacy of diet and physical activity in preventing weight gain and elevations in other cardiovascular risk factors. Women were enrolled from 1992 to 1994, randomized to either lifestyle intervention or an assessment-only control, and followed over 54 months. Beginning in 1994, carotid ultrasound scans were offered to all participants, and were obtained in 453 women (85%).

To be eligible for enrollment, women had to be aged 44 to 50 years, premenopausal (defined as less than 3 months amenorrhea in the 6 months before the screening interview), and have a diastolic blood pressure (DBP) <95 mm Hg, BMI between 10 and 34, fasting glucose <7.77 mmol, low density lipoproteins (LDLs) between 2.07 and 4.14 mmol, and total cholesterol between 3.26 and 6.72 mmol. Exclusion criteria included hysterectomy, hormone therapy, and use...
of antihypertensive, lipid-lowering, insulin, thyroid, or psychotropic medications. Further details of the clinical trial are available in previous publications.25-27

Both the clinical trial and the carotid exams were approved annually by the Institutional Review Board of the University of Pittsburgh. All participants provided written consent.

Clinical Measures
Baseline clinical measures included systolic blood pressure (SBP), DBP, weight, BMI (kg/m²), waist circumference, percent body fat (from dual energy x-ray absorptiometry scanner) and fasting total cholesterol, high density lipoproteins (HDLs), LDL2, HDL3, LDLs, triglycerides, apoprotein B, and glucose. Dietary intake (kilocalories per day) was assessed by questionnaire.28 Leisure time physical activity (kilocalories in the past week) was measured by interview.29 Serum insulin was assessed at the 6-month follow-up visit rather than at baseline. Standard enzymatic assays were used to measure total serum cholesterol, HDLs, triglycerides, and glucose; LDL was estimated by the Friedewald equation; apoprotein B was determined via turbidimetric measurement; and serum insulin was determined by radioimmunoassay. Smoking was categorized as current, former, or never. Additional details of the clinical measures are available in previous publications.25-27

At each follow-up visit and at the time of their scan, women were assessed for hormone therapy use (any in the year previous) and hysterectomy, and categorized as pre-, peri-, or postmenopausal (perimenopausal indicates missing a bleeding cycle or taking hormone therapy for 3 to 11 cycles in the last year; postmenopausal indicates no bleeding cycle or taking hormone therapy for ≥12 consecutive cycles, or hysterectomy). Because most women remained premenopausal at scan time, menopausal status was dichotomized as premenopausal versus peri/postmenopausal for analyses.

Carotid Ultrasound Measures
Carotid arteries were examined using an ultrasound scanner (Toshiba SSA-270A) equipped with a 5-MHz linear array imaging probe. With participant in supine position, arteries were viewed in transverse and longitudinal projections. For the common carotid artery (CCA) segment, both near and far walls were examined 2 cm proximal to the bifurcation (bulb). For the bulb area and internal carotid artery (ICA), measurements were taken of the far walls only (because near walls cannot be consistently visualized). Digitized images were used to trace the medial-adventitial and intima-lumen interfaces across 1-cm lengths and compute IMT for each segment.30 Average IMT was calculated from the mean of both the right and left carotid arteries of the 3 segments (8 locations total).

Our IMT reliability study (n=15)30 reported correlation coefficients of 0.96 between sonographers and 0.99 between readers for average IMT, and was >0.87 for individual segments across sonographers and readers.

Statistical Analysis
Descriptive measures are summarized as mean±SD unless otherwise noted. Intervention and control groups were compared using standard t tests, Wilcoxon tests, and χ² tests. Analyses are reported on the pooled population, given that risk factors were collected before the intervention and to maximize available data.

Pearson correlation coefficients were calculated between normally distributed cardiovascular risk factors and each IMT measure. Spearman correlations were calculated for nonnormal risk factors. To determine which traditional cardiovascular risk factors were independently associated with each IMT segment, variables with a P value of ≤0.15 in univariate correlations were included in multivariable linear regression models. More parsimonious multivariable models were identified with a stepwise selection procedure, using P<0.05 as the selection cutoff. Models were adjusted for age, intervention group, and menopausal status at the time of the scan. The 3 segment models (ie, CCA, bulb, and ICA) suggested that risk factor associations with IMT varied according to the IMT measurement location.

To formally test these observed differences, a single model using all 3 IMT measures was developed. Because the 3 IMT measures taken from each woman were not independent, a repeated measures model was used. This modeled each IMT value (ie, 3 lines of data per person: 1 for CCA IMT, 1 for bulb IMT, and 1 for ICA IMT) as a function of risk factors and a 3-level variable indicating location. The correlation between each woman’s IMT values, which is accounted for in the repeated measures model, was modeled using a compound symmetric correlational structure.31 Covariates in the repeated measures model included all significant multivariable regression predictors, IMT measurement location, and examined possible location by risk factor interaction effects. Significant interaction effects would signify segment-specific effects. All analyses were implemented using the SAS system for Windows (version 8.2; SAS Institute).

Results
The women scanned were similar in clinical characteristics to those not scanned (data not shown). Clinical characteristics for the 453 participants are presented in Table 1 and were similar across control and intervention groups.

Scans were done ~2.7 years (range 0.7 to 7.6 years) after the baseline visit when 84% of all subjects were still premenopausal. There were no significant differences in IMT measures between treatment groups, thus the groups were combined for remaining analyses (Table 2). Mean average IMT was 0.68 mm in the pooled group; IMT was highest in the bulb and lowest in the ICA segment. For each measure, results include only participants for whom both the right- and left-sided IMT measurement was available. Those with missing IMT (n=9) were similar in available data to those included (data not shown).

Univariate Associations
In unadjusted correlations, average IMT was positively and significantly (P<0.15) associated with typical cardiovascular risk factors of age, weight, BMI, waist circumference, percent body fat, SBP, DBP, lipid measures (ie, cholesterol, LDL, apoprotein B, and triglycerides), insulin, and physical activity, whereas HDL and HDL2 showed a negative association. Examining arterial segments individually, IMT at all 3 locations was positively associated with baseline weight, BMI, and waist circumference. Additionally, higher CCA IMT was associated with older age and higher SBP, DBP, percent body fat, apoprotein B, and insulin; higher bulb IMT was associated with higher SBP, DBP, percent body fat, apoprotein B, and insulin; higher bulb IMT was associated with higher SBP, DBP, percent body fat, lipid measures, insulin and physical activity, and lower HDL and HDL2; higher ICA IMT was associated with older age, higher lipid measures and insulin, and lower HDL. In unadjusted bivariate analyses, significant positive associations were found between average IMT and smoking, hormone therapy, and hysterectomy. By segment, significant positive associations were found between CCA IMT and smoking, hormone therapy and hysterectomy, and between bulb IMT and smoking. Correlations between IMT across the 3 locations were moderate (range r=0.32 to 0.43).

Multivariable Associations
Linear regression models were adjusted for intervention group, age, and menopausal status at scan time. Risk factors showing significant univariate correlations with IMT (listed above) were included in respective models for average IMT and each location. Significant independent predictors of greater average IMT
were higher weight, SBP, age, and current smoking (Table 3). Higher weight, SBP, age, and hysterectomy were independently associated with higher CCA IMT. Current smoking, weight, and SBP were positively associated with bulb IMT. Higher apoprotein B was associated with higher ICA IMT. Comparing individual covariate percents of variation in each model (ie, adjusted R2), it was evident that weight was the largest contributor to average and CCA IMT, SBP was most prominent for bulb IMT, and apoprotein B was nearly the sole contributor to ICA IMT. Additionally, age at scan was 1.03% and 1.34% of the “other” category for average and CCA IMT, respectively (Figure).

Thus, differential effects could be seen across arterial locations. For example, a 10mm Hg increase in SBP would produce a 0.008 mm increase in CCA IMT, a 0.022 mm increase in bulb IMT, and no significant change in ICA IMT, although these comparisons do not take into account variations in wall thickness. To test the statistical significance of these observed differences, a single repeated measures linear model using all 3 IMT measures (thus accounting for within-woman and between-women variability in IMT) was used to examine segment-specific associations.

**Repeated Measures**

The repeated measures model included significant multivariable predictors from the previous models (weight, SBP, smoking, apoprotein B, and hysterectomy), control variables (intervention group, menopausal status, and age), segment location (CCA, bulb, ICA), and risk factor by segment interaction terms. This was a single model with IMT as the dependent variable and multiple lines of data per woman, corresponding to each IMT segment. As expected, significant main effect associations with IMT were found across the 3 locations for weight (β=0.007 per 5 kg, P<0.001), SBP (β=0.008 per 10 mm Hg, P=0.008), current smoking (compared with never smoked β=0.037, P=0.005), and apoprotein B (β=0.006 per 0.1 g/L, P=0.01) with a trend for age at

**TABLE 1. Participant Characteristics (n=453)*

<table>
<thead>
<tr>
<th>Characteristics at Baseline</th>
<th>Characteristics at the Time of the Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>46.9±1.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67.3±9.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1±3.3</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>78.5±8.5</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>33.1±4.6</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>110.3±12.7</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>68.2±8.2</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.90±0.63</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.53±0.33</td>
</tr>
<tr>
<td>HDL2, mmol/L</td>
<td>0.28±0.16</td>
</tr>
<tr>
<td>HDL3, mmol/L</td>
<td>1.23±0.22</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>2.97±0.57</td>
</tr>
<tr>
<td>Apoprotein B, g/L</td>
<td>0.74±0.16</td>
</tr>
<tr>
<td>Triglycerides, mmol/L†</td>
<td>0.80 (0.60–1.10)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.4±0.4</td>
</tr>
<tr>
<td>Insulin, pmol/L‡</td>
<td>99.8±54.8</td>
</tr>
<tr>
<td>Dietary intake, kcal/d</td>
<td>904 (504–1817)</td>
</tr>
<tr>
<td>Physical activity, kcal/wk</td>
<td>0 (0–805)</td>
</tr>
</tbody>
</table>

**TABLE 2. Intima Media Thickness**

<table>
<thead>
<tr>
<th>Location</th>
<th>Control</th>
<th>Intervention</th>
<th>Pooled Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common carotid artery, mm</td>
<td>451</td>
<td>0.68±0.08</td>
<td>0.67±0.08</td>
</tr>
<tr>
<td>Carotid bifurcation, mm</td>
<td>452</td>
<td>0.74±0.14</td>
<td>0.74±0.16</td>
</tr>
<tr>
<td>Internal carotid artery, mm</td>
<td>447</td>
<td>0.62±0.10</td>
<td>0.62±0.10</td>
</tr>
<tr>
<td>Average, mm</td>
<td>444</td>
<td>0.68±0.08</td>
<td>0.67±0.08</td>
</tr>
</tbody>
</table>
scan ($\beta=0.02$ per 5 years, $P=0.08$). Results were the same for never and former smokers. Significant interaction effects (signifying segment-specific effects) were found between location and SBP ($P=0.02$), smoking ($P=0.01$), and apoprotein B ($P=0.02$). Specifically, simple effects estimates (Table 4) revealed a greater effect of weight and age in the CCA than in the ICA, and a greater effect for apoprotein B in the ICA than in the CCA. Smoking and SBP appeared to have an effect that was specific to the bulb segment. In addition, individual estimates showed significant segment associations between weight and apoprotein B and bulb location. There were no main or interaction effects for hysterectomy, which was likely a surrogate for age in this sample. Thus, significant and unique associations between baseline risk factors and segments exist even after accounting for the correlation in IMT at all 3 locations.

### Discussion

Our research shows that cardiovascular risk factors may differentially influence atherosclerosis in carotid arterial segments in healthy middle-aged women. Further, repeated measures regression, which has not been used in previous studies of carotid segments, showed that these segment-specific associations were statistically significant when locations were directly compared.

Results of this study build on our previous findings of carotid atherosclerosis, where we reported on average IMT in a subset (n=292) of the study sample. Similar associations between cardiovascular risk factors and average IMT were found. Additionally, segment-specific analyses were possible with the larger sample size.

### Segment-Specific Carotid IMT

Unique to our study were the examination of IMT by carotid segment in healthy, primarily premenopausal women and the examination of risk factor and location interactions. Variations in methodology, including use of older women or heart clinic patients, make others’ results of IMT by carotid segment challenging to compare. Nonetheless, similarities between our results and those previously published include positive associations of SBP and body composition with CCA and bulb IMT, lipids associated with bulb and ICA IMT, and smoking with bulb IMT. In contrast, neither lipids nor smoking were found to be associated with CCA IMT in our analyses. Nor did we find older age associated with higher IMT across segments, although this may be because of our narrow age range. Further, others have generally found the segments similarly associated with cardiovascular risk factors, whereas we found distinct differences in risk factor and segment associations. Examining more diseased populations may have made it harder for others to tease out unique associations.

### Theories on Differential IMT

Various mechanical and biochemical pathways and the risk factors themselves are most certainly acting in conjunction...
and possibly synergistically to determine the development and progression of atherosclerosis at any given site.\textsuperscript{23,24} The moderate level of IMT we found in the CCA (compared with the ICA and bulb) is likely due to the lower shear wall force\textsuperscript{18,19,32} that is consistent with the laminar blood flow\textsuperscript{33} in this location. Its association with higher weight is consistent with research on sheer stress and lipids regarding age\textsuperscript{19} in 18.3% of our sample (posthoc; plaque defined as a distinct area protruding into the vessel lumen with >50% thickness than surrounding area). The influence of shear stress on the extracellular factors of hypertension, such as increased pressure,\textsuperscript{16} decreases in vasoactive agents,\textsuperscript{17} and inflammatory effects,\textsuperscript{14} would have the largest impact on the bulb segment. Research on increased residence time of blood\textsuperscript{36} and adherence of platelets and macrophages to the arterial wall,\textsuperscript{40} which influence lipid infiltration and plaque formation, is consistent with our positive association between apoprotein B and bulb IMT. The increased residence time might also make the bulb more susceptible to the toxic components of cigarette smoke.\textsuperscript{15}

Several study limitations should be mentioned. First, the wide time range between baseline and scan visits may have influenced results, although 91% of participants had scans within 1 SD of the mean interval. Posthoc, we substituted “time interval” and “age at baseline” for the covariate “age at scan” in regression models. Longer time interval replaced older age at scan as a significant predictor of higher average and CCA IMT, thus the model adequately adjusted for time. Second, risk factors explained only 3% to 9% of IMT segment variance, so other atherosclerotic mechanisms and pathways should continue to be examined. However, given the healthy status, young age, and low levels of IMT in our sample, the link between specific IMT segments and risk factors are noteworthy and comparable to the 12% to 22% segment variance found by Wei et al\textsuperscript{13} whose sample included diabetic subjects. Further, the enrollment exclusion criteria (ie, requiring normal to high-normal ranges for DBP, BMI, glucose, and lipids) likely reduced the effects of these risk factors and potentially increased the relative contribution of other risk factors (eg, hysterectomy). Third, there may have been some influence of the intervention that was not detected in our analyses because the clinical trial was successful in minimizing increased weight and LDL that traditionally occur with menopause.\textsuperscript{25–27} Lastly, we examined a healthy, homogeneous sample of women, who may not be representative of the general population and thus may have underestimated associa-

### Table 4. Linear Repeated Measures Model of Intima-Media Thickness*

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Common Carotid Artery</th>
<th>Carotid Bifurcation</th>
<th>Internal Carotid Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention group</td>
<td>–0.012, 0.033, 0.008</td>
<td>–0.007, –0.027, 0.014</td>
<td>–0.006, –0.027, 0.015</td>
</tr>
<tr>
<td>Peri/Postmenopausal</td>
<td>0.002, –0.034, 0.030</td>
<td>0.010, –0.022, 0.043</td>
<td>0.001, –0.031, 0.034</td>
</tr>
<tr>
<td>Age at scan, 5 y</td>
<td>0.027†, 0.001, 0.053</td>
<td>0.018, –0.008, 0.044</td>
<td>0.006, –0.020, 0.032</td>
</tr>
<tr>
<td>Weight, 5 kg</td>
<td>0.008‡, 0.003, 0.014</td>
<td>0.009§, 0.004, 0.015</td>
<td>0.004, –0.002, 0.009</td>
</tr>
<tr>
<td>SBP, 10 mm Hg</td>
<td>0.006, –0.002, 0.015</td>
<td>0.016§, 0.008, 0.025</td>
<td>0.002, –0.006, 0.011</td>
</tr>
<tr>
<td>Apoprotein B, 0.1 g/L</td>
<td>–0.000, –0.007, 0.007</td>
<td>0.007†, 0.001, 0.014</td>
<td>0.011§, 0.005, 0.018</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.010, –0.025, 0.046</td>
<td>0.077§, 0.041, 0.112</td>
<td>0.024, –0.011, 0.060</td>
</tr>
<tr>
<td>current vs never</td>
<td>–0.001, –0.038, 0.035</td>
<td>0.073§, 0.036, 0.110</td>
<td>0.023, –0.013, 0.060</td>
</tr>
<tr>
<td>current vs former</td>
<td>0.031, –0.032, 0.094</td>
<td>–0.023, –0.086, 0.040</td>
<td>0.001, –0.063, 0.064</td>
</tr>
</tbody>
</table>

*This was a single model with IMT as the outcome measures and included all of the covariates below, IMT measurement location, and location by risk factor interaction effects.

†P<0.05.
‡P<0.01.
§P<0.001.
CI=95%.
tions. Future research should include other groups for a better understanding of segment-specific associations and their influences on clinical events.

In conclusion, cardiovascular risk factors traditionally associated with overall mean carotid IMT may differentially influence wall thickening of the carotid artery segments in middle-aged healthy women, even after adjusting for other cardiovascular risk factors and wall thickness in all 3 segments.

Acknowledgments
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References
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