Thicker carotid intima layer and thinner media layer in subjects with cardiovascular diseases
An investigation using noninvasive high-frequency ultrasound

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Abstract

Background: The thickness of the arterial intima increases and that of the media decreases with increasing age and degree of atherosclerosis. Separate estimates of the individual intima and media layers might therefore be more appropriate than the commonly used method estimating the combined intima-media thickness (IMT).

Methods and results: One hundred consecutive 70-year-old subjects from the PIVUS study were investigated. Separate estimates of the thickness of the carotid artery intima and media wall layers were carried out noninvasively using 25 MHz high-frequency ultrasound. Subjects with a diagnosis of cardiovascular disease (CVD), coronary heart disease (CHD), myocardial infarction (MI) or stroke had a significantly thicker intima layer (all \( P < 0.0001 \)) and a thinner media layer (all \( P < 0.05 \)) than healthy subjects. The intima/media thickness ratio also differed significantly between subjects with and without a diagnosis of CVD (0.43 ± 0.20 versus 0.75 ± 0.48, \( P = 0.0002 \)). Subjects with hypertension or hyperlipidemia also had a thicker carotid intima than subjects without these diagnoses (\( P < 0.0005 \) for both). None of the corresponding intima + media thickness values differed significantly. Similar results were obtained in women and men.

Conclusion: Separate assessment of carotid artery intima and media thickness using noninvasive high-frequency ultrasound appears to be of potential value, as a striking difference in intima thickness and the intima/media thickness ratio was found between subjects with and without CVD.

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Keywords: Carotid artery wall; Intima-media thickness (IMT); Cardiovascular disease; Atherosclerosis; High-frequency ultrasound
methods may have other advantages none of them estimate the intima thickness and media thickness separately. However, by intravascular high-frequency ultrasound (30 MHz) separate estimates of media thickness can be obtained [7]. Recently, a noninvasive high-frequency ultrasound instrument (25 MHz) has been used to obtain separate estimates of intima and media thickness with reliable reproducibility (Fig. 1) [10,11]. The aim of this study was to evaluate the usefulness and validity of a technique using high-frequency ultrasound for separately assessing the thickness of artery wall.
intima and media layers and evaluating the intima thickness and intima/media thickness ratio in 70-year-old subjects, with and without a history of CVD, who were participating in a health screening programme (the PIVUS study).

1. Subjects and methods

One hundred consecutive subjects from a cohort of 1016 randomly selected persons, all aged 70 years, living in the Uppsala community and participating in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS, see http://www.medsci.uu.se/pivus/pivus.htm) study, were included in this evaluation. All subjects underwent a physical examination and recorded any medical history of CHD (myocardial infarction (MI), angina pectoris, coronary bypass or balloon angioplasty), heart failure, stroke, hypertension, diabetes and hyperlipidemia. CVD was defined as the presence of CHD, heart failure, stroke or hypertension. CHD outcomes were defined by International Classification of Diseases, 10th revision (ICD-10) codes I20–I25, stroke by ICD codes I60–I69, hypertension by ICD codes I10–I15, diabetes by ICD codes E10–E14 and hyperlipidemia by ICD code E78. The duration of hypertension, diabetes and hyperlipidemia was estimated as years of treatment for these disorders. Each subject gave informed consent to the study, and the study was approved by the Local Ethics Committee, Uppsala University, Sweden.

1.1. Measurements of artery wall thickness

Before beginning the ultrasound imaging, subjects rested quietly for at least 15 min. Carotid total wall thickness and the thickness of the individual artery layers were assessed non-invasively using high-resolution ultrasonographic equipment (Osteoson® Minhost GmbH, Meudt, Germany), fitted with a broad-banded probe with 25 MHz center frequency. The method was validated for the estimation of total artery wall thickness and media layer thickness in non-atherosclerotic superficial arteries in pigs, and a comprehensive description of the methodology is available in that study [10]. Briefly, the scan converter enables the image to be frozen at a selected scan-time (2 s) and the unit permits two-dimensional data acquisition, presenting the results as scans A and B. About 128 lines of echo data were detected as an A-echo signal, sampled by an eight-bit analog-to-digital converter, converted by scanning to a rectangular format and viewed as B-mode images on a 32-colour scale monitor. Image resolution is approximately 0.07 mm axially along the ultrasonographic beam and the depth of focus is in the range of 13.5–14.5 mm in front of the tip of the probe. The system recognizes objects of about 0.015 mm in size, and the software-driven cursors permit a minimal digital display of 0.02 mm.

The left common carotid artery (LCCA) was examined at the point of the strongest pulse, in front of the sternocleidomastoid muscle, with the subjects sitting in an upright position.

### Table 1: Cardiovascular diseases in the study group by gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>CVD ( n=39 )</th>
<th>CHD ( n=11 )</th>
<th>MI ( n=7 )</th>
<th>Stroke ( n=7 )</th>
<th>Hypertension ( n=30 )</th>
<th>Diabetes ( n=7 )</th>
<th>Hyperlipidemia ( n=12 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Yes 21 (37.5)</td>
<td>No 35 (62.5)</td>
<td>Yes 7 (12.5)</td>
<td>No 49 (87.5)</td>
<td>No 51 (91)</td>
<td>No 10 (18)</td>
<td>No 46 (82)</td>
</tr>
<tr>
<td>Women</td>
<td>Yes 18 (41)</td>
<td>No 26 (59)</td>
<td>Yes 4 (9)</td>
<td>No 40 (91)</td>
<td>No 42 (95.5)</td>
<td>No 7 (16)</td>
<td>No 37 (84)</td>
</tr>
<tr>
<td>Total</td>
<td>Yes 39 (39)</td>
<td>Yes 11 (11)</td>
<td>Yes 7 (7)</td>
<td>Yes 7 (7)</td>
<td>Yes 30 (30)</td>
<td>Yes 7 (7)</td>
<td>Yes 12 (12)</td>
</tr>
</tbody>
</table>

* CVD: all cardiovascular diseases combined including coronary heart disease (CHD), heart failure, stroke and hypertension.
* CHD: including myocardial infarction, angina pectoris, coronary bypass or balloon angioplasty.
* MI: myocardial infarction.
position and looking straight ahead. The three-layer image showed the pulsating artery near wall and the artery lumen. Ten B scans (point estimates) were carried out and measurements of the thickness of the whole arterial wall and its layers were performed off-line. Means of the 10 measurements were calculated and used in the analysis. The total thickness of the carotid wall was measured from the leading edge of the adventitia to the far edge of the intima. Measurements of the adventitia and intima were made using only the brightest echoes from leading edge to far edge, and the thickness of the media layer was measured as the distance between the two brightest echoes. The coefficient of variation (CV%), calculated from double estimates (based on the mean of 5 measurements each) in 20 subjects, was about 2.4% for total carotid wall, 4.2% for media layer and about 8% for the intima layer. The values for artery wall layer given in this study were mean values based on 10 or more measurements. All ultrasonographic assessments were performed by the same investigator (K.R.-M.), who was blinded to the clinical data of the study subjects.

1.2. Statistical methods

Comparison of numerical variables between groups was carried out using the two-sample t-test or Wilcoxon Rank Sum test, depending on the result of the test for normality based on the Shapiro–Wilk W test. The Chi square test was used to compare distributions of categorical variables. Spearman-rank correlation tests were used to investigate correlations between variables. The CV%, based on duplicate measurements, was estimated according to the formula: $\text{CV\%} = 100 \times \left( \frac{\text{S.D.}}{\text{mean}} \right)$, and standard deviation (S.D.) was estimated as: $\sqrt{\sum d^2/2n}$, where $d$ = difference between duplicate measurement values and $n$ = number of duplicate determinations [12].

2. Results

The study group comprised 100 participants, 56 men and 44 women. The prevalence of cardiovascular disease was fairly similar in men and women. There was a tendency for a higher prevalence of diabetes mellitus in men than in women (10.7% versus 2%, respectively, $P=0.08$), Table 1.

2.1. Association between risk factors for cardiovascular disease and carotid artery total wall thickness, media thickness, intima thickness and intima/media thickness ratio

In the whole study population ($n=100$), carotid intima thickness was positively associated with weight, body mass index (BMI), waist circumference, hip circumference and waist/hip ratio (all $P<0.005$), as well as with duration of hypertension, duration of hyperlipidemia and number of cigarettes/week (all $P<0.05$). Intima thickness was also associated (borderline significance) with duration of smoking ($r=0.19$, $P=0.057$). The intima/media thickness ratio was positively associated with BMI, duration of hyperlipidemia and number of cigarettes/week (all $P<0.05$), Table 2. There were no significant associations between media thickness and risk factors for CVD. The thickness of the whole carotid wall was positively associated with weight, waist circumference and waist/hip ratio (all $P<0.05$), Table 2.

2.2. Carotid total wall thickness, media thickness, intima thickness and intima/media thickness ratio by type of cardiovascular disease

Compared with healthy subjects, subjects with a diagnosis of CVD, CHD, MI or stroke had a thinner mean carotid media layer (all $P<0.05$), a thicker intima layer (all $P<0.0001$) and a substantially (74–195%) higher intima/media thickness ratio (all $P<0.0005$), Table 3. Intima + media thickness and total artery wall thickness did not differ significantly in any of the CVD category groups from that in healthy subjects. Compared with healthy subjects, patients with hypertension or hyperlipidemia had thicker intima layers and a higher intima/media thickness ratio ($P<0.0005$ and $P<0.05$, respectively), Table 3. Separate analyses for men and women with regard to type of cardiovascular disease (Tables 4 and 5) revealed results fairly similar to those in the combined study.

Table 2: Associations between risk factors for CVD and total carotid wall, media thickness, intima thickness and intima/media ratio in the study group ($n=100$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total wall</th>
<th>Media</th>
<th>Intima</th>
<th>Intima/media ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$</td>
<td>$r_s$</td>
<td>$P$</td>
</tr>
<tr>
<td>Weight</td>
<td>0.26</td>
<td>0.01</td>
<td>0.09</td>
<td>0.4</td>
</tr>
<tr>
<td>BMI</td>
<td>0.12</td>
<td>0.3</td>
<td>−0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>Waist</td>
<td>0.21</td>
<td>0.03</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Hip</td>
<td>0.10</td>
<td>0.3</td>
<td>−0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.20</td>
<td>0.003</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Years of hypertension</td>
<td>−0.0002</td>
<td>0.9</td>
<td>−0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>Years of hyperlipidemia</td>
<td>0.06</td>
<td>0.6</td>
<td>−0.17</td>
<td>0.1</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>−0.03</td>
<td>0.7</td>
<td>−0.07</td>
<td>0.5</td>
</tr>
<tr>
<td>Number of cigarettes/week</td>
<td>−0.09</td>
<td>0.4</td>
<td>−0.15</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$r_s$: Spearman-rank correlation coefficient.
Table 3

Carotid total artery wall, media thickness, intima thickness and intima/media (I/M) ratio estimated by high-frequency ultrasound by presence and type of CVD when compared with healthy subjects with no CVD (n = 61).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± S.D.</th>
<th>P</th>
<th>Mean ± S.D.</th>
<th>P</th>
<th>Mean ± S.D.</th>
<th>P</th>
<th>Mean ± S.D.</th>
<th>P</th>
<th>Difference (%)</th>
<th>P</th>
<th>I + M (mean ± S.D.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total wall</td>
<td>All CVD</td>
<td>0.4</td>
<td>&lt;0.05</td>
<td>Intima</td>
<td>P &lt;0.0001</td>
<td>74</td>
<td>&lt;0.0005</td>
<td>0.60</td>
<td>0.15</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>Yes (n = 39)</td>
<td>0.72 ± 0.20</td>
<td>0.38 ± 0.16</td>
<td>0.75 ± 0.48</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No (n = 61)</td>
<td>0.75 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Intima</td>
<td>Yes (n = 11)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I/M ratio</td>
<td>Yes (n = 11)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Difference</td>
<td>Yes (n = 11)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I + M</td>
<td>Yes (n = 11)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stroke</td>
<td>Yes (n = 17)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>Yes (n = 30)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
<td>Yes (n = 7)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyperlipidemia</td>
<td>Yes (n = 21)</td>
<td>0.72 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.43</td>
<td>0.20</td>
<td>0.62</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subjects with diabetes and hyperlipidemia were compared with subjects without those disorders.

a All CVD: all cardiovascular diseases combined including coronary heart disease (CHD), heart failure, stroke and hypertension.

b CHD: including myocardial infarction, angina pectoris, coronary by-pass or balloon angioplasty.

c MI: myocardial infarction.

d Including subjects treated with lipid lowering medications.

e Percentage difference from mean values in healthy subjects.

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Subjects with diabetes and hyperlipidemia were compared with subjects without those disorders.

<table>
<thead>
<tr>
<th>CVDa</th>
<th>Diabetes</th>
<th>Hyperlipidemia</th>
<th>Hypertension</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CVD</td>
<td>All CVD</td>
<td>All CVD</td>
<td>All CVD</td>
<td>All CVD</td>
</tr>
<tr>
<td>Yes (n = 38)</td>
<td>No (n = 52)</td>
<td>Yes (n = 21)</td>
<td>No (n = 37)</td>
<td>Yes (n = 34)</td>
</tr>
<tr>
<td>0.65 ± 0.2</td>
<td>0.67 ± 0.2</td>
<td>0.34 ± 0.2</td>
<td>0.39 ± 0.1</td>
<td>0.21 ± 0.1</td>
</tr>
<tr>
<td>0.66 ± 0.06</td>
<td>0.67 ± 0.03</td>
<td>0.15 ± 0.04</td>
<td>0.14 ± 0.01</td>
<td>0.31 ± 0.1</td>
</tr>
<tr>
<td>0.66 ± 0.1</td>
<td>0.67 ± 0.1</td>
<td>0.29 ± 0.1</td>
<td>0.39 ± 0.1</td>
<td>0.47 ± 0.1</td>
</tr>
</tbody>
</table>

Subjects with hyperlipidemia were compared with subjects without that disorder.

a All CVD: all cardiovascular diseases combined including coronary heart disease (CHD), heart failure, stroke and hypertension.

b CHD: including myocardial infarction, angina pectoris, coronary by-pass or balloon angioplasty.

c MI: myocardial infarction.

d Including subjects treated with lipid lowering medications.
Cardiovascular diseases are often interrelated with each other. It is known that about 62% of strokes and 49% of MI group, Table 3. However, analysis by gender revealed that men had significantly higher mean values for carotid total wall thickness, media thickness and intima thickness than women (all $P < 0.05$). Furthermore, men with diabetes melilites had a thiner carotid media layer and men with hypertensive had a thicker carotid intima layer than the equivalent layers in healthy men ($P < 0.05$ for both), Table 4. The intima/media thickness ratio was also significantly higher in men with hypertension, diabetes or hyperlipidemia than in healthy men (all $P < 0.05$), whereas no significant differences were found in women (Tables 4 and 5).

3. Discussion

The main finding of this study was that separate estimates of carotid intima thickness, media thickness, and the resulting intima/media thickness ratio, obtained noninvasively using high-frequency (25 MHz) ultrasound, resulted in highly significant differences between 70-year-old subjects with and without various forms of CVD. These findings were consistent and fairly similar in women and men. In contrast, no significant differences were found when estimates of intima + media thickness were used. Thus, our findings suggest that separate estimates of intima and media thickness and the use of the intima/media thickness ratio could be a valuable tool in the evaluation of pathophysiological changes in the artery wall.

According to our previous validation study, this high-frequency ultrasound equipment can be reliably used to estimate total wall and media layer thickness, but is less suitable for estimation of intima layer thickness in non-atherosclerotic arteries [10]. However, this study, which included subjects with atherosclerotic disease, indicates that assessing the intima thickness by itself results in clear, significant differentiation between subjects with and without atherosclerosis. Furthermore, subjects with a diagnosis of hypertension, stroke or hyperlipidemia also had thicker carotid intima layers than healthy subjects, suggesting additional potential value from estimation of the thickness of the intima layer using high-frequency ultrasound.

The conventional noninvasive assessment and monitoring of changes in the whole artery wall, i.e. the IMT, estimated using 7–8 MHz frequency ultrasound, has been widely used in epidemiological and clinical studies and has revealed acceptable associations with prevalence [2] and risk of CVD [3,4]. However, when Adams et al. compared IMT and coronary angiography in 350 subjects, carotid IMT was only weakly correlated to severity of coronary artery disease ($r = 0.23–0.26$) and not specific to sensitive enough to identify subjects with and without significant coronary artery disease [5]. This could be explained by the fact that an increase in IMT is predominantly the result of an increase in the thickness of the intima layer [13]. However, the thickness of the media layer is also included in the IMT estimates. The simultaneous reduction in media thickness with increasing atherosclerosis will therefore diminish the sensitivity of the IMT assessment. Furthermore, the specific changes in the thickness of the media layer cannot be identified by 7–8 MHz frequency ultrasound because of the systematic overestimation of the intima layer and the systematic underestimation of the media layer associated with low resolution [14].

The clinical interpretation of low-resolution ultrasonography IMT measurements has been further questioned by Gamble et al. who showed, by means of a validation study, that IMT measurements of carotid arteries (using 7–8 MHz frequency ultrasonography), in situ in cadavers best corresponded with histologically ascertained total artery wall thickness, i.e. including adventitia, rather than with the intima + media complex per se [15]. These findings may in part explain that values for the carotid intima + media thickness in our study were smaller than those from conventional IMT using 7–8 MHz frequency ultrasound. Our values are, however, very similar to those obtained in men of similar age [16] and in postmenopausal women [17] using 10 MHz frequency ultrasound. As with our data, the IMT in men is significantly thicker than that in women when estimated using 7–8 MHz frequency ultrasound [18,19]. Although sex differences in body fat distribution may account for part of this difference, the association between carotid IMT and waist/hip ratio is similar for both sexes [19].

A reduction in the thickness of the media layer of the carotid artery with aging [11], as well as in subjects with CVD or CHD, as indicated in this report, may appear inconsistent with the abundant data of an increase in carotid IMT associated with the presence of CVD, as assessed by 7–8 MHz frequency probes [20]. However, Gussenhoven et al. used high-frequency intravascular ultrasound to demonstrate that artery media thickness is reduced by atherosclerosis [7]. The extent of medial thinning seems to be inversely related to the extent of intimal thickening, indicating that medial thinning is an essential part of the atherosclerosis process [7]. Morphometric studies in elderly subjects have also shown an age-related increase in intima and decrease in media thickness, particularly in the carotid arteries, as well as inverse correlations between media thickness and degree of stenosis in the aorta, and the carotid, coronary and cerebral arteries [6]. Thus, present and previous [11] results with regard to changes in intima and media thicknesses, using noninvasive assessments, are totally in agreement with results based on the advanced intravascular ultrasound (IVUS) [7] and those from morphometric studies [6]. Other noninvasive methods, i.e. MR, duplex ultrasound or computed tomography [8] do not estimate the intima and media thickness separately.

The substantial difference in intima/media thickness ratio between subjects with CVD and healthy subjects found in this study clearly results from divergent changes in carotid intima and media thickness associated with arterial disease.

3.1. Study limitations

Cardiovascular diseases are often interrelated with each other. It is known that about 62% of strokes and 49% of MI...
are caused by high blood pressure [21]. The CVD categories presented in this study, i.e. CHD, MI, stroke and hypertension, may therefore overlap to include other CVD. Because of the relatively small sample of 100 subjects, further analyses involving the exclusion of subjects with combined CVD diagnoses were not performed. The inclusion of individuals who were all of the same age precluded estimation of the effect of aging on the thickness of the artery wall layers within the study group. Conclusions are based on the larger groups, values for subgroups are given to illustrate that the method seems robust. Further, the substantial and significant differences also for subgroups and risk factors, despite the small sample size, support the strength of the method.

4. Conclusions

We demonstrated substantial differences in the thicknesses of the artery intima and media layers between groups of subjects with and without CVD. We suggest, therefore, that the use of noninvasive high-frequency ultrasound for separately estimating artery intima and media thickness could be a valuable tool for assessing/monitoring changes caused by aging, atherosclerosis and the effects of medical interventions on the artery wall.

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References