Acute Change in Vascular Tone Alters Intima-Media Thickness

Dick H.J. Thijssen, Ralph R. Scholten, Inge C.L. van den Munckhof, Nathalie Benda, Daniel J. Green, Maria T.E. Hopman

Abstract—Atherosclerosis is a lifelong process involving artery wall thickening. Increased wall thickness has been widely adopted as a preclinical surrogate marker of atherosclerosis. A prerequisite for such a surrogate marker is that it is a structural characteristic of the vessel wall that is not subject to acute changes. The purpose of this study was to examine the acute effects of vasodilator drug administration on wall thickness of the carotid and superficial femoral arteries. High-resolution ultrasound was used to examine carotid and femoral artery diameters and wall thickness in 15 young (25±4 years of age) and 15 older (70±6 years of age) healthy men who were administered sublingual glyceryl trinitrate. Diameter and wall thickness were collected before and across a 10-minute period after glyceryl trinitrate administration. Glyceryl trinitrate induced a significant increase in carotid and femoral artery diameter and a decrease in wall thickness in both young and older men (both P<0.001). The latter was significantly larger than in young men (both P<0.01). The changes in carotid artery wall thickness in both young (35±23 μm) and older men (71±46 μm) approximate those considered prognostically relevant. Collectively, our data suggest that vasodilator drug administration induces a rapid and marked decrease in wall thickness, which mirrors conduit artery vasodilation in both young and older men. This finding confirms the presence of acute changes in wall thickness and has important implications for future studies that assess artery wall characteristics as a surrogate measure of atherosclerosis. (Hypertension. 2011;58:240-246.)

Key Words: atherosclerosis ■ aging ■ wall characteristics ■ cardiovascular risk ■ arterial remodeling

Atherosclerosis plays a central role in the development of cardiovascular disease.1–4 Conduit arterial wall thickening, particularly of the carotid artery, is regarded as a surrogate marker of the atherosclerotic process and has been used in intervention trials as a primary outcome measure.1–8 Noninvasive ultrasound is widely used to assess wall thickness, sometimes referred to as intima-medial thickness (IMT), which is an established independent predictor for future cardiovascular events.4,6,7,9

Clinical trials of pharmacological interventions (eg, statins, lipid lowering therapy, and vitamin C) typically study changes in IMT across a 1- to 4-year period.10–13 Wall thickness has, therefore, widely been considered a structural vascular characteristic that changes slowly over long time periods, consistent with progression of the atherosclerotic process.14,15 For this reason, assessment of conduit artery wall thickness is typically performed without controlling for factors that influence vascular tone (eg, exercise, and medication use).16,17 Although acute changes in vascular smooth muscle tone may theoretically alter artery wall thickness, no previous studies have examined this possibility.

Therefore, we examined the acute effect of vascular smooth muscle relaxation (using an NO donor) on wall thickness of a predominantly elastic (carotid) and muscular (superficial femoral) artery. We hypothesized that smooth muscle relaxation would lead to acute and systematic changes in IMT.

Age is associated with structural changes in the vasculature, including arterial wall thickening.4,18,19 Acute changes in carotid and superficial femoral artery wall thickness after glyceryl trinitrate (GTN) administration were compared between healthy young and older men. We hypothesized that older men, because of the atherosclerotic process, would demonstrate significantly smaller changes in artery wall thickness.

Methods

Subjects
Fifteen healthy young men (≤30 years of age) and 15 older healthy sedentary men (≥60 years of age) were recruited from the community (Table 1). Subjects were no more than recreationally active (≤4 hours per week of exercise). We excluded smokers and subjects who were diagnosed with diabetes mellitus (type I or II), rheumatoid

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Table 1. Subject Characteristics of Healthy Young (n=15) and Older Subjects (n=15)

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Young Men (n=15)</th>
<th>Older Men (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>25±4</td>
<td>70±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74±9</td>
<td>80±12</td>
<td>0.048</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8±2.2</td>
<td>25.1±2.9</td>
<td>0.014</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.85±0.05</td>
<td>0.91±0.06</td>
<td>0.008</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118±6</td>
<td>126±7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75±6</td>
<td>78±6</td>
<td>0.13</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.53±0.86</td>
<td>5.66±0.94</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.95±0.39</td>
<td>1.16±0.38</td>
<td>0.18</td>
</tr>
<tr>
<td>High-density lipoproteins, mmol/L</td>
<td>1.28±0.27</td>
<td>1.44±0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Low-density lipoproteins, mmol/L</td>
<td>2.83±0.74</td>
<td>3.70±0.82</td>
<td>0.008</td>
</tr>
</tbody>
</table>

The P value refers to an unpaired t test between young and older men. Blood analysis in young men was performed in 11 subjects.

Experimental Design
Young healthy men attended our laboratory on 4 occasions. In a randomized order, we examined changes in wall thickness and diameter of the carotid and superficial femoral arteries after a sublingual dose of GTN (ie, an NO donor) or a control spray to examine the normal variation in wall thickness. Older participants reported 2 times to our laboratory to examine changes in diameter and wall thickness of the carotid and superficial femoral arteries (in a randomized order) to GTN only. Measurements were performed after a 4-hour fast and 12 hours of abstinence from caffeine, chocolate, alcohol, kiwi, and vitamin supplements. None of the participants gave written informed consent before entering the study. The study was approved by the medical ethics committee of the Radboud University Nijmegen Medical Centre and conformed to the standards set out by the Declaration of Helsinki.

Experimental Procedures
On day 1, we measured physical characteristics, such as height, weight, body mass index, and waist:hip ratio. Blood pressure was measured in duplicate using a manual sphygmomanometer with the participants placed in a comfortable chair. The mean of the 2 measurements was used for further analysis. A venous blood sample was taken for analysis of fasting levels of total cholesterol, triglycerides, and high- and low-density lipoproteins.

After the 20-minute resting period, baseline wall thickness and diameter of the common carotid or superficial femoral artery were measured using high-resolution echo ultrasonography with a 7.5-MHz linear array transducer (Picas, Pie Medical Benelux, Maastricht, The Netherlands). The left common carotid artery was measured 2 cm proximal to the bulbus and the right superficial femoral artery was measured 2 to 3 cm distal to the bifurcation. For the baseline assessment, we measured artery diameter and wall thickness in 3 series of 6 consecutive cardiac cycles. Subsequently, participants received sublingual administration of a single dose of GTN (400 µg, G Pohl-Boskamp GmbH and Co KG, Hohenlockstedt, Germany) or the control spray, which consisted of a mouth spray (400 µg Aquafresh, GlaxoSmithKline). Participant and sonographer were blinded to administration of GTN or the control spray, although both sprays were mint flavored. Conduit artery wall thickness and diameter were measured continuously for a period of 10 minutes after administration, with data from the last 6 consecutive cardiac cycles of each minute the focus of further analysis. The order of the testing days was randomized.

Conduit Artery Diameter and Wall Thickness Analysis
Ultrasound analysis was performed with a multiarray echo tracking system (ART-Laboratory, Esaote Europe BV, Eindhoven, The Netherlands), based on high-resolution echo tracking technology, which is described in detail elsewhere. Briefly, a longitudinal 2D ultrasound image of the artery was taken, with both arterial walls clearly displayed. A region of interest was taken of the intima-media thickness. Data were analyzed in real time across the cardiac cycle. This method is robust and reliable, operator independent, and shows a high accuracy for the estimation of conduit artery diameter and wall thickness. Assessment of arterial diameter and wall thickness across the cardiac cycle takes into account variation between diastole and systole. This approach differs from other studies in which wall thickness measures are confined to those assessed during diastole.

In our laboratory, a day-to-day coefficient of variation of 8.6% and 3.6% was found for the carotid artery diameter and IMT (young men, n=15). The coefficients of variation for the superficial femoral artery wall thickness and diameter were 14.0% and 3.8%. This is in line with other studies and indicate that our study (2-sided)
Table 2. Carotid and Superficial Femoral Artery Characteristics of Healthy Young (n=15) and Older Men (n=15) at Baseline and Maximum Changes After Administration of Glyceryl Trinitrate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Men (n=15)</th>
<th>Older Men (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>6.4±0.6</td>
<td>8.1±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in diameter, mm</td>
<td>0.4±0.2</td>
<td>0.3±0.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Change in diameter, %</td>
<td>6.0±2.6</td>
<td>4.0±2.8</td>
<td>0.051</td>
</tr>
<tr>
<td>Baseline wall thickness, µm</td>
<td>393±60</td>
<td>778±155</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in wall thickness, µm</td>
<td>35±23</td>
<td>71±46</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in wall thickness, %</td>
<td>8.7±5.3</td>
<td>9.2±5.2</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline wall:lumen ratio</td>
<td>0.062±0.008</td>
<td>0.096±0.013</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in wall:lumen ratio</td>
<td>0.008±0.003</td>
<td>0.012±0.005</td>
<td>0.08</td>
</tr>
<tr>
<td>Change in wall:lumen ratio, %</td>
<td>13.5±5.3</td>
<td>12.1±5.9</td>
<td>0.54</td>
</tr>
<tr>
<td>Superficial femoral artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>7.2±0.8</td>
<td>8.3±1.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in diameter, mm</td>
<td>0.6±0.3</td>
<td>0.3±0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Change in diameter, %</td>
<td>8.7±3.8</td>
<td>3.8±2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline wall thickness, µm</td>
<td>382±59</td>
<td>588±202</td>
<td>0.002</td>
</tr>
<tr>
<td>Change in wall thickness, µm</td>
<td>36±15</td>
<td>67±27</td>
<td>0.001</td>
</tr>
<tr>
<td>Change in wall thickness, %</td>
<td>9.3±3.6</td>
<td>11.7±5.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Baseline wall:lumen ratio</td>
<td>0.053±0.008</td>
<td>0.072±0.023</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in wall:lumen ratio</td>
<td>0.008±0.002</td>
<td>0.010±0.004</td>
<td>0.17</td>
</tr>
<tr>
<td>Change in wall:lumen ratio, %</td>
<td>14.8±3.0</td>
<td>14.0±5.6</td>
<td>0.64</td>
</tr>
</tbody>
</table>

The P value refers to an unpaired t test between young and older men.

possession 97% power to detect a clinically relevant IMT change of 30 µm. We have also calculated the cross-sectional area of the wall.13,27

Statistics

Statistical analyses were performed using Excel (Microsoft Office Excel 2007) and SPSS (17.0, SPSS Inc, Chicago, IL). A 2-way repeated-measures ANOVA (time × intervention) was used to examine whether changes in carotid and superficial femoral artery diameter and wall thickness across the 10 minute period (“time”) differed between GTN and the control spray (“intervention”). When a significant change was found, a post hoc analysis, using the Sidak test for multiple comparisons, was performed. Unpaired t tests were used to examine differences in baseline characteristics between young and older men. Another 2-way repeated-measures ANOVA (time × group) was used to compare changes in diameter and wall thickness across the 10-minute period (“time”) between young and older men (“group”). A Pearson correlation coefficient was used to examine the relation between the cross-sectional area of the arterial wall before and after intervention to examine whether the volume of the arterial wall was maintained (“conservation of mass”). All of the data are expressed as mean±SD unless otherwise indicated. Statistical significance (2-sided P) was set at <0.05.

Results

Carotid Artery Characteristics in Young Men: GTN Versus Control Spray

Baseline carotid artery diameter and wall thickness were not different between testing days (t test: P=0.48 and 0.93, respectively). Carotid artery diameter and wall thickness changed significantly after GTN (both P<0.001), with a significant interaction between the impact of GTN versus the control intervention on diameter and wall thickness (P<0.001 and 0.007, respectively; Figure 1). Post hoc analysis revealed a gradual increase in artery diameter after GTN (P<0.001), which was mirrored by a simultaneous decrease in carotid artery wall thickness (P<0.001). The control intervention induced no changes in carotid artery diameter or wall thickness (P=0.16 and 0.23, respectively; Figure 1). A high correlation was found between the cross-sectional area of the carotid arterial wall before versus after GTN or control spray (r=0.96 and 0.99, respectively; both P<0.001).

Superficial Femoral Artery in Young Men: GTN Versus Control Spray

Resting superficial femoral artery diameter and wall thickness were not different between testing days (t test: P=0.49 and 0.61, respectively; Table 2). Superficial femoral artery diameter and wall thickness significantly changed during the intervention, with a significant interaction between both interventions for the changes in diameter and wall thickness (Figure 2). GTN induced a gradual increase in superficial femoral artery diameter (P<0.001), whereas wall thickness showed a simultaneous decrease (P<0.001; Figure 2). The control spray induced no change in diameter and wall thickness.
thickness ($P=0.10$ and 0.49, respectively; Figure 2). A strong correlation was found in superficial femoral artery cross-sectional area before and after GTN and or control ($r=0.97$ and 0.98, respectively; both $P<0.001$).

GTN in Carotid and Superficial Femoral Artery: Young Versus Older Men

Young and older men showed no differences in diastolic blood pressure, triglycerides, or high-density lipoprotein, whereas older men demonstrated higher body mass index, waist:hip ratio, systolic blood pressure, cholesterol, and low-density lipoprotein (Table 1), as well as a larger carotid and superficial femoral artery diameter and wall thickness (Table 2). GTN induced a significant increase in superficial femoral and carotid arterial diameters and a decrease in wall thickness in older men (Figure 3). A correlation was found between the cross-sectional area of the carotid and superficial arterial walls before versus after GTN ($r=0.98$ and 0.99, respectively; both $P<0.001$).

Carotid Artery

The rates of increase (Figure 4) and peak change (Table 2) in carotid artery diameter after GTN were similar between groups. In contrast, the absolute change (Figure 4) and maximum decrease (Table 2) in wall thickness to GTN in older men were significantly larger than observed in young men. In addition, the decrease (Figure 4) and peak change (Table 2) in superficial femoral artery wall thickness to GTN in older men were larger than in young men.

Superficial Femoral Artery

The GTN-mediated increase (Figure 4) and the maximum change (Table 2) in superficial femoral artery in older men were significantly smaller than observed in young men. In addition, the decrease (Figure 4) and peak change (Table 2) in superficial femoral artery wall thickness to GTN in older men were larger than in young men.

Discussion

Our primary findings are as follows: (1) wall thickness decreased in response to a vasodilator substance in the carotid and superficial femoral arteries of young men because of smooth muscle relaxation; (2) the decrease in wall thickness occurred gradually, mirroring dilation of the lumen; (3) changes in wall thickness in the carotid and superficial femoral arteries were also present in older men; and (4) magnitude of decrease in wall thickness in older men was larger than that observed in their young peers. Collectively, conduit artery smooth muscle relaxation leads to an acute decrease in arterial wall thickness according to normal physiological principles. More importantly, our observations strongly indicate that future IMT studies should control for factors that induce acute variability in vascular tone, because this importantly alters wall thickness.

Assessment of conduit artery wall thickness is widely regarded as a structural index of the vessel wall, frequently used as a measure of subclinical atheroma and an independent predictor for future cardiovascular risk. A recent meta-analysis revealed that a $100-\mu$m change in carotid artery wall thickness is associated with an 18% change in cardiovascular risk. We found that the mean reduction in conduit artery wall...
thickness under GTN application in older men (in both arteries) approached this value (71 and 67 μm; Table 2). These acute changes in conduit artery wall thickness have, therefore, important consequences for studies that adopt IMT as a surrogate measure of future cardiovascular risk.\textsuperscript{10–13} It is notable that recent measurement guidelines do not address the possibility of acute changes in wall thickness,\textsuperscript{16,17,28} but our results raise the possibility that conduit artery wall thickness can change rapidly in humans. In a recent study, we observed a 20% increase in carotid and superficial femoral artery wall thickness after only 60 days of bed rest,\textsuperscript{3} a value \textasciitilde75 times greater than the expected effect of aging, per se. We suggest that the assumption that IMT represents an immutable characteristic of long-term atherosclerotic change should be reassessed. Our current data demonstrate that artery wall thickness should be regarded as a dynamic part of the vascular system that is able to change acutely and over the short term.\textsuperscript{3} A plausible explanation for the changes in wall thickness to GTN relates to the conservation of arterial wall mass. The strong correlation of the arterial wall cross-sectional area before and after GTN supports this hypothesis. However, future studies should further explore this mechanism and potential age-related differences.

Our data indicate that GTN induced a 10% decrease in carotid and superficial femoral artery wall thicknesses, which was independent of age, artery type (elastic versus muscular), and baseline wall thickness. This observation conceals the fact that older men demonstrated a significantly larger wall thickness at baseline and a significantly larger decrease in wall thickness in absolute terms compared with the younger subjects. The latter observation is of clinical importance, because it indicates the magnitude of error in IMT or conduit artery wall thickness assessment. In addition, the error in prediction of future cardiovascular events may increase in subjects with a larger baseline wall thickness or those at higher age. Indeed, a positive correlation was found between resting wall thickness and the GTN-induced decrease in wall thickness across both arteries and both groups (r=0.54; P<0.001). It is unclear whether older subjects with established cardiovascular disease or risk factors would be capable of demonstrating such large degrees of wall thickness change in response to dilator substances. Indeed, we specifically examined plaque-free segments of the carotid and superficial femoral arteries in older subjects. Whether a segment with plaque formation or more advanced atherosclerotic disease would demonstrate similar responses is unknown. Because
arterial diameter change in response to acute vasodilator stimuli (eg, flow-mediated vasodilation) and baseline wall thickness (IMT) both possess cardiovascular prognostic capacity, it might be speculated that the artery wall thickness response to a standard stimulus (ie, vasodilator drug) may provide a useful index of the impact of atherosclerotic cardiovascular disease progression and future risk.

Limitations

There are some limitations to this experiment. Carotid artery diameter and wall thickness measurements were stopped after 10 minutes, and a plateau in wall thickness change may not have been achieved. This suggests that the marked decrease in carotid artery wall thickness that we observed may be an underestimation of the true capacity for wall thickness change. Secondly, we examined changes in wall thickness in healthy male subjects without the (clinical) presence of atherosclerotic plaques. It is unclear whether similar changes in wall thickness are present in subjects with multiple cardiovascular risk factors. Also, premenopausal and postmenopausal women may demonstrate different responses compared with men, because estrogen levels influence vascular tone and vascular function.

Perspectives

We found, for the first time, that conduit artery wall thickness changes markedly in response to vasodilation, which is independent of baseline wall thickness and artery studied, whereas the absolute decrease in arterial wall thickness is significantly larger in older men. Future studies examining conduit artery wall thickness should, therefore, consider that changes in smooth muscle vascular tone alter conduit arterial wall thickness.

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