Arterial stiffness, but not endothelium-dependent vasodilation, is related to a low Ankle-Brachial index

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study

Lars Lind

Department of Medicine, Uppsala University Hospital, Uppsala, Sweden

Summary

Background: Arterial compliance and endothelium-dependent vasodilation are two characteristics of the vessel wall. In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study we studied the relationships between arterial compliance and endothelium-dependent vasodilation versus the Ankle-Brachial index (ABI), a clinically used index of peripheral artery disease.

Methods: In the population-based PIVUS study (all aged 70), arterial compliance was determined by ultrasound as the distensibility of the carotid artery and the stroke volume to pulse pressure (SV/PP) ratio by echocardiography, while endothelium-dependent vasodilation was assessed by the invasive forearm technique with acetylcholine (EDV) and brachial artery ultrasound (FMD) in 519 subjects in whom the Ankle-Brachial index was investigated.

Results: After adjustments for gender and Framingham risk score, distensibility in the carotid artery and the SV/PP ratio were significantly reduced in subjects with a reduced ABI (<0.9) in both legs (n = 15, P = 0.0006 and P = 0.0003, respectively). Endothelium-dependent vasodilation was not significantly related to a reduced ABI.

Conclusion: A reduced arterial compliance, but not endothelium-dependent vasodilation, was related to a low ABI in both legs after adjustment for major risk factors, suggesting that atherosclerosis in the leg arteries is associated with arterial compliance also in other parts of the vasculature.

Introduction

Peripheral vascular disease (PVD) is a common disorder of the elderly. In the epidemiological setting it is most often defined either according to a history of claudicatio intermittens or by a low Ankle-Brachial index (ABI) (Tsai et al., 2001; Murabito et al., 2003; Lee et al., 2004; Guerrero et al., 2005; Feringa et al., 2006). The underlying cause is in the majority of cases a stenosis in the lower limb arteries due to atherosclerotic lesions, and it has repeatedly been shown that a low ABI could predict the major cardiovascular disorders in prospective cohort studies (Tsai et al., 2001; Murabito et al., 2003; Lee et al., 2004; Guerrero et al., 2005; Feringa et al., 2006).

Arterial compliance and endothelium-dependent vasodilation are two other major characteristics of the arteries. These two vascular features are both related to the main cardiovascular risk factors (Creager et al., 1990; Linder et al., 1990; Celermajer et al., 1992; Roman et al., 1992; Johnstone et al., 1993; Laurent et al., 1994; Lehmann et al., 1998), and have both the ability to predict future cardiovascular outcomes in prospective studies (Blacher et al., 1998; Schachinger et al., 2000; London et al., 2001; Perticone et al., 2001; Lind et al., 2004).

Both a reduced arterial compliance and an impaired endothelium-dependent vasodilation have been previously been described in patients with overt PVD (Yataco et al., 1999; Taniwaki et al., 2001; Cheng et al., 2002; Poredos et al., 2003; Silvestro et al., 2003). However, there are sparse data on how these two indices of vascular function relates to ABI in a general population. In the present study, we investigated the relationships between arterial compliance and endothelium-dependent vasodilation versus ABI, using data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)
study (Lind et al., 2005), with the hypothesis that both arterial compliance and endothelium-dependent vasodilation were impaired in those with a low ABI.

Materials and methods

This section has previously been given in detail (Lind et al., 2005, 2006).

Subjects

Eligible were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were randomly chosen from the register of community living. 1016 subjects participated giving a participation rate of 50%. In the last consecutive 519 subjects of the sample, ABI was measured. The study was approved by the Ethics Committee of the University of Uppsala. Signed informed consent was given by all subjects.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. An arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators.

Blood pressure was measured by a calibrated mercury sphygmomanometer. Fasting blood glucose and lipids were measured by standard techniques and from these values the Framingham risk score was calculated (Wilson et al., 1998). Basic characteristics of the total sample are given in Table 1, since the ABI subsample did not differ from the total sample in terms of the major characteristics. Amongst those with a reduced ABI, 13% reported a history of myocardial infarction and 5% reported a history of stroke.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke and 9% diabetes mellitus. Almost half the cohort reported any cardiovascular medication (45%), with antihypertensive medication being the most prevalent (32%). Fifteen percent reported use of statins, while insulin and oral antiglycemic drugs were reported in 2% and 6%, respectively.

Ankle-Brachial index

Brachial systolic blood pressure was measured in the brachial artery by the mean of three recordings with a mercury sphygmomanometer recording the arm with the highest value. Ankle systolic blood pressure was measured in anterior tibial arteries in both legs using Doppler to identify the systolic blood pressure when a cuff was placed around the lower part of the leg was deflated following arrest of blood flow in the lower leg. All blood pressure measurements were performed after at least 30 min rest in the supine position. The ABI was calculated as the ratio between the brachial and ankle systolic blood pressure for each leg separately.

The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden). A mercury in-silastic strain-gauge was placed at the upper third of the forearm, which rested comfortably slightly above the level of the heart. The strain-gauge was connected to a calibrated plethysmograph. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mm Hg by a rapid cuff inflator. Evaluations of FBF were made by calculations of the mean of at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. No more than one attempt to insert the cannula in each arm was allowed. Resting FBF was measured 30 min after cannula

Table 1 Major cardiovascular risk factors, prevalence of cardiovascular medication and disorders in subjects with a normal Ankle-Brachial index (ABI > 0.9), and in subjects with a low ABI in one or both legs.

<table>
<thead>
<tr>
<th></th>
<th>Normal ABI</th>
<th>Low ABI in one leg</th>
<th>Low ABI in both legs</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>482</td>
<td>22</td>
<td>15</td>
<td>0.81</td>
</tr>
<tr>
<td>Females (%)</td>
<td>48</td>
<td>50</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>27.0 ± 3.2</td>
<td>26.1 ± 4.2</td>
<td>27.9 ± 4.8</td>
<td>0.43</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>149 ± 22</td>
<td>157 ± 25.75</td>
<td>172 ± 23.18</td>
<td>0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78 ± 10</td>
<td>78 ± 10</td>
<td>82 ± 9</td>
<td>0.41</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.2 ± 0.8</td>
<td>3.4 ± 1.0</td>
<td>3.4 ± 0.9</td>
<td>0.53</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum triglycerides (mmol l⁻¹)</td>
<td>1.3 ± 0.6</td>
<td>1.4 ± 0.7</td>
<td>1.6 ± 0.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol l⁻¹)</td>
<td>5.4 ± 1.5</td>
<td>6.5 ± 3.7**</td>
<td>5.8 ± 1.9</td>
<td>0.011</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>7</td>
<td>32**</td>
<td>33**</td>
<td>0.0001</td>
</tr>
<tr>
<td>Framingham risk score</td>
<td>11.1 ± 3.3</td>
<td>12.7 ± 3.5**</td>
<td>13.9 ± 3.0**</td>
<td>0.0001</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>32</td>
<td>41</td>
<td>60</td>
<td>0.07</td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>16</td>
<td>9</td>
<td>27</td>
<td>0.37</td>
</tr>
<tr>
<td>Antidiabetic treatment (%)</td>
<td>6</td>
<td>18</td>
<td>13</td>
<td>0.15</td>
</tr>
<tr>
<td>History of myocardial infarction (%)</td>
<td>8</td>
<td>9</td>
<td>20</td>
<td>0.22</td>
</tr>
<tr>
<td>History of stroke (%)</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Values given are mean ± SD or %. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.
During infusion of 50 \(\mu g\) min\(^{-1}\) of Acetylcholine and SNP were used. EDV was defined as FBF divided by resting FBF. EIDV was defined as FBF during imaging 2–3 cm above the elbow (Acuson, 7-MHz transducer, Acuson, Mountain View, CA, USA) according to the recommendations of the International Brachial Artery Task Force. Depths and gains settings were optimized to identify the lumen to vessel wall interface. The subject rested in the supine position for at least 30 min before the first scan and remained supine during the evaluation. Blood flow increase was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mmHg above systolic blood pressure. When the cuff was rapidly deflated 5 min later, the artery was scanned continuously for 90 s and recorded on a super-VHS videotape for later analysis at the diameter. The diameter was manually measured at the peak of the R-wave at baseline and at 30, 60 and 90 s following cuff deflation. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus diameter at rest divided by the diameter at rest.

**Pulse wave analysis**

A micromanometer tipped probe (Sphygmacor; Pulse Wave Medical Ltd, West Ryde, Australia) was applied to the surface of the skin overlying the radial artery and the peripheral radial pulse wave was continuously recorded. The mean values of around 10 pulse waves were used for analyses. Recordings were regarded as satisfactory if the variations in the systolic peak and the diastolic peak were 5% or below. Only three attempts to achieve a satisfactory recording were allowed. The maximal systolic peak and the reflected waves were identified by the calculations of the first and second derivative of the pulse curve. Based on transfer functions, aortic systolic and diastolic blood pressure were calculated from the radial recordings with the Sphygmacor software. Central pulse pressure (PP) was calculated by central systolic minus central diastolic blood pressure.

In the present study only data from the highest doses of Acetylcholine and SNP were used. EDV was defined as FBF during infusion of 50 \(\mu g\) min\(^{-1}\) of Acetylcholine minus resting FBF divided by resting FBF. EIDV was defined as FBF during infusion of 10 \(\mu g\) min\(^{-1}\) of SNP minus resting FBF divided by resting FBF.

**The brachial artery ultrasound technique**

The brachial artery was assessed by external B-mode ultrasound imaging 2–3 cm above the elbow (Acuson, 7-MHz linear transducer, Acuson, Mountain View, CA, USA) according to the recommendations of the International Brachial Artery Task Force. Depths and gains settings were optimized to identify the lumen to vessel wall interface. The subject rested in the supine position for at least 30 min before the first scan and remained supine during the evaluation. Blood flow increase was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mmHg above systolic blood pressure. When the cuff was rapidly deflated 5 min later, the artery was scanned continuously for 90 s and recorded on a super-VHS videotape for later analysis at the diameter. The diameter was manually measured at the peak of the R-wave at baseline and at 30, 60 and 90 s following cuff deflation. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus diameter at rest divided by the diameter at rest.

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**Echocardiography and Doppler**

A comprehensive two-dimensional and Doppler echocardiography was performed with an Acuson XP124 cardiac ultrasound unit (Acuson). A 2-5-MHz transducer was used for the majority of the 2-D-, M-mode and Doppler examinations. This investigation was carried out in 97% of the subjects. Presence of stenosis or regurgitations in the mitral and aortic valves was recorded by use of colour and continuous Doppler.

**Carotid artery compliance**

The diameter of the common carotid artery (CCA) of the right side 1–2 cm proximal of the bifurcation was measured at its maximal diameter in systole and the minimal diameter in diastole by an Acuson XP124 cardiac ultrasound unit (Acuson) with a 2-5-MHz transducer. The distensibility of the CCA was calculated as the percent change in diameter maximum to minimum in relation to the minimal diameter in diastole divided by the central pulse pressure obtained by pulse wave analysis.

**Reproducibility**

We have previously shown the reproducibility (coefficient of variation, CV) at repeated measurements to be 3% for baseline brachial artery diameter and 29% for flow-mediated vasodilation (Lind et al., 2000) and the corresponding CVs for Endothelium-dependent vasodilation in forearm resistance vessels and Endothelium-independent vasodilation in forearm resistance vessels to be 8–10% (Lind et al., 1998). For the compliance measures carotid artery distensibility and Stroke volume to pulse pressure ratio the CVs were 21% and 14%, respectively.

**Statistics**

Non-normally distributed variables (such as EDV, EIDV, CCA distensibility, SV/PP-ratio and FMD) were log-transformed to achieve a normal distribution. ANOVA and ANCOVA were used to compare means between groups. The Bonferroni correction was used for post hoc analysis. The chi-square test was used to evaluate differences in proportions. Two-tailed significance values were given with \(P<0.05\) regarded as significant. The statistical programme package StatView (SAS Inc., Cary, NC, USA) was used.
Results

The mean ABI in the population was 1.15 (0.16 SD) in the right leg and 1.14 (0.16) in the left leg. These mean values are in practice identical and normal in that age group.

When a reduced ABI was defined as <0.90, 22 subjects showed a reduced ABI in one of the legs and another 15 subjects showed an impaired ABI in both legs.

If the sample was divided into these two groups together with a group with a normal ABI in both legs, the two groups with a reduced ABI showed higher SBP and Framingham risk score and an increased prevalence of smoking compared with subjects with a normal ABI. Levels of DBP, BMI, LDL or HDL–cholesterol, triglycerides were not different between the groups, while fasting glucose was significantly increased in the group with a low ABI in one leg only (see Table 1 for details).

The prevalences of antihypertensive medication, statin use and antidiabetic treatment were not significantly between the groups. Neither were any significant differences between the groups seen regarding history of myocardial infarction and stroke (Table 1).

When the sample was divided into these three groups, a significant difference in EIDV was seen between the groups (P = 0.013) after adjustment for gender, with similarly reduced levels in both groups with a reduced ABI compared to the normal group. EDV and FMD were not different between the three groups (see Table 2 for details).

Both of the arterial compliance measurements were significantly different between the groups (P < 0.004 for both) after adjustment for gender. In both the case of CCA distensibility and the SV/PP ratio, reductions were only seen in the group with a reduced ABI in both legs when compared to the normal group (P = 0.0006 and 0.0003, respectively, Fig. 1). The differences between the groups were still significant also after addition of the Framingham score as independent variable to the models (P = 0.02 for CCA distensibility and P = 0.01 for the SV/PP ratio).

Discussion

The present study showed two measurements of arterial compliance to be reduced in subjects with a low ABI in both legs after adjustment for major cardiovascular risk factors, while endothelium-dependent vasodilation was not altered in subjects with a low ABI.

Arterial compliance

The ABI is a commonly used variable to assess the occurrence of peripheral artery disease in epidemiological studies, as well as in the clinical setting. A low ABI has repeatedly been shown to predict future cardiovascular events and 0.90 is usually used as a cut-off limit (Taniwaki et al., 2001; Lee et al., 2004). In the present study, a reduced arterial compliance was mainly seen in those with a low value in both legs.

A reduced arterial compliance has previously been recorded in patients with overt PVD, as evaluated by compliance in the carotid or femoral artery (Taniwaki et al., 2001; Cheng et al., 2002). However, no clear effect of PVD on arterial compliance was seen when evaluated by pulse wave analysis (Duprez et al., 2002). However, no clear effect of PVD on arterial compliance was seen when evaluated by pulse wave analysis (Duprez et al., 2002).
In the present study endothelium-dependent vasodilation was not related to ABI, neither when measured in the brachial artery, nor when evaluated in the forearm resistance arteries.

This in contradiction to previous studies showing a reduced FMD in patients with overt PVD (Yataco et al., 1999; Poredos et al., 2003; Silvestro et al., 2003). It has recently been shown that FMD is of limited value in subjects with reduced arterial compliance (Witte et al., 2005; Lind, 2007), and the fact that arterial compliance as such was related to a low ABI limits the power to detect an effects of a low ABI on FMD. Previous studies using FMD were performed in subjects with overt PVD possibly representing profoundly more severe cases of PVD than found in the present population-based study, in which only a minority of those with a low ABI reported any claudication symptoms. In these claudication patients FMD measurements seem to be of value, as they predict future cardiovascular events (Brevetti et al., 2003; Gokce et al., 2003).

No previous study has reported acetylcholine-mediated dilation in the forearm vessels in subjects with low ABI. Since one study reported a reduced endothelium-dependent vasodilation in the leg resistance vessels in PVD patients (Weiss et al., 2002), it may be that only the affected vascular bed is influenced by the atherosclerotic process.

Contrary to the finding with EDV, EIDV was reduced in subjects with a low ABI. This indicates that the vasodilatory mechanisms not influenced by the endothelium, such as the vascular smooth muscle cells in the forearm resistance arteries are impaired in subjects with a low ABI. Thus, vasodilation in resistance arteries is a problem in subjects with a low ABI and might contribute to the symptoms seen in peripheral artery disease.

Clinical implications

CCA distensibility, the SV/PP ratio and ABI have all been shown to predict CV events in population studies. Thus, from this perspective all three CV markers seem to be of value. Nevertheless, since ABI is an easily obtainable measure in the clinical practice this measure might be the preferred one, since it also is of value for screening of PVD.

Limitations

The present sample is limited to Caucasians aged 70. Caution should therefore be made to draw conclusions to other ethnic and age groups.

The PIVUS study had a moderate participation rate. However, an analysis of non-participants showed the present sample to be fairly representative of the total population regarding most cardiovascular disorders and drug intake.

EIDV was only assessed by one of the methods for practical and ethical reasons not to prolong the investigation procedure. We have previously shown that EIDV evaluated by SNP infusion in the brachial artery and nitroglycerine provoked change in brachial artery diameter are closely related (Lind et al., 2000), so additional measurements of EIDV would probably not add substantial information to the study.

It must furthermore be pointed out that none of the measurements of arterial compliance or endothelium-dependent vasodilation were carried out in the leg vessels.

Thus, we evaluated vascular characteristics in vascular beds being remote from those being the basis for the measurement of the low ABI. Other results may have emerged if arterial compliance or endothelium-dependent vasodilation had been evaluated in the vasculature of the legs.

Another limitation is that aortic pulse wave velocity, the most commonly used index of arterial compliance, was not evaluated in the present cohort.

Only six subjects reported claudication, and due to the small number no statistical evaluation of vascular function in subjects with overt claudication could be performed.

In conclusion, an impaired arterial compliance, but not endothelium-dependent vasodilation, was related to a low ABI in both legs after adjustment for major risk factors, suggesting that atherosclerosis in the leg arteries influences arterial compliance also in other parts of the vasculature.

Acknowledgement

The outstanding work at the endothelium laboratory performed by Nilla Fors, Jan Hall, Kerstin Marttala and Anna Stenborg in the collection and processing of the data is highly acknowledged.
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