Impaired endothelial function has been suggested as an important cause of cardiovascular disease, but its best method of assessment and its clinical value for risk prediction remain to be determined. A major function of the arterial endothelium is to regulate vasodilation by secreting nitric oxide. Hence, endothelium-dependent vasodilation (EDV) was originally evaluated as the vasmotor response to acetylcholine (which stimulates nitric oxide release) infused in a coronary artery.\(^1\) Endothelial function measured with that method was related to the extent of coronary atherosclerosis,\(^2\) but its use is restricted to subjects undergoing coronary angiography for clinical reasons. Subsequently, a number of less invasive techniques for measuring EDV have been developed.

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Its noncoronary successor, the so-called invasive forearm technique, uses infusion of acetylcholine in the brachial artery, and the increase in forearm blood flow is taken as a measure of EDV. This technique evaluates mainly EDV in forearm resistance arteries. A reduced EDV has been found in patients with coronary heart disease, hypertension, hypercholesterolemia, or diabetes mellitus.\(^3\)--\(^7\)

Another method to assess EDV is the ultrasound-based method evaluating flow-mediated vasodilation (FMD) in the brachial artery during hyperemia.\(^8\)\(^9\) This technique evaluates EDV in a conduit artery. An attenuated FMD has been observed in patients with coronary heart disease and in those with major risk factors.\(^10\)\(^11\)

With the use of applation tonometry of the radial artery to capture the peripheral pulse pressure waveform, the reduction in the reflected waves induced by adrenergic \(\beta\)-2 receptor agonist stimulation\(^12\)\(^13\) is a measure of EDV in resistance arteries. A reduced response to \(\beta\)-2 agonists has been demonstrated in patients with hypercholesterolemia, coronary heart disease, hypertension, and diabetes mellitus.\(^12\)\(^16\)

An impaired EDV in the coronary arteries has been associated with risk of cardiovascular events in 2 samples of coronary artery disease patients.\(^3\)\(^17\) Impaired EDV in both forearm resis-
tance vessels\textsuperscript{7,18,19} and the brachial artery\textsuperscript{20--24} has also been associated with risk of cardiovascular events in different patient groups. Recently, an association of low FMD with risk of cardiovascular events was also observed in the population-based setting.\textsuperscript{25,26} In addition, a low hyperemic blood flow, indicating a microcirculatory disturbance, was found to be a predictor of future events in a patient study.\textsuperscript{27} Furthermore, endothelial release of tissue-type plasminogen activator has recently been found to predict cardiovascular events in coronary patients.\textsuperscript{28}

We initiated the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study with the primary aim of evaluating the capacity of these 3 different tests of EDV in the peripheral circulation to predict subsequent cardiovascular events in a community-based sample of >1000 men and women 70 years of age. Because we had observed that all 3 vasoreactivity tests were related to coronary risk as evaluated by the Framingham risk score,\textsuperscript{15} we hypothesized that all 3 tests of vasoreactivity would be related to the 5-year risk of MACEs during 5 years of follow-up.

**Methods**

**Sample**

All subjects who were 70 years of age and living in the community of Uppsala, Sweden, were eligible. The subjects were chosen from the register of community living, and were invited in a randomized order. The subjects received an invitation by letter within 2 months of their 70th birthday. Of the 2025 subjects invited, 1016 subjects participated, giving a participation rate of 50.1%. The baseline investigation was started in April 2001.\textsuperscript{18} The excluding of 67 participants with a myocardial infarction and 28 subjects with a stroke before baseline resulted in a sample of 921 participants to be evaluated in a prospective fashion. The study was approved by the Ethics Committee of Uppsala University, and the participants gave informed consent.

**Baseline Clinical Investigations**

The participants were asked to answer a questionnaire about their medical history, smoking habits, and regular medication. All participants were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. Blood pressure was measured by a calibrated mercury sphygmomanometer in the noncannulated arm to the nearest 1 mm Hg after at least 30 minutes of rest, and the average of 3 recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques. High-sensitivity C-reactive protein was measured by latex-enhanced turbidimetry on a Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL). The Framingham risk score published in 2008 was calculated as described previously.\textsuperscript{29}

**Vascular Tests**

After recordings of height, weight, and abdominal and hip circumference, an arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. During the investigation, the subjects were supine in a quiet room maintained at a constant temperature. The total investigation took 4 hours. The invasive forearm model with intrabrachial infusion of acetylcholine to assess EDV and sodium nitroprusside to assess endothelium-independent vasodilation (EIDV) was carried out first, followed by FMD and finally the pulse-wave--based technique. At least 30 minutes passed between the different tests. Flow-mediated vasodilation was assessed as the change in brachial artery diameter measured by ultrasound before and after 5 minutes of circulatory arrest of the forearm with the cuff placed below the elbow. The pulse-wave--based test is based on the determination of the reflected pulse wave assessed by aplanation tonometry of the radial artery following stimulation with terbutaline. We also assessed common carotid artery intima-media thickness (IMT) in the far wall by ultrasound. The vascular tests are described in detail in Table I in the online-only Data Supplement.\textsuperscript{15}

The coefficients of variation (CVs) for EDV and EIDV were 8% to 10%.\textsuperscript{30} The EDV technique was not used in participants on regular warfarin because of expected problems with bleeding (n = 32). In another 106 subjects, cannulation of the brachial artery failed or some other technical error occurred, leaving 794 (87%) with a valid test. The CV for baseline brachial artery diameter was 3%, and the CV for FMD was 29%.\textsuperscript{31} The artery could not be visualized in a proper way in 27 participants, resulting in valid recordings in 899 participants (97%).

The CV for the change in reflection index (ΔRI) was 9.4%.\textsuperscript{32} After 3 cases of fainting after terbutaline injection in subjects with frequent premature ventricular beats or atrial fibrillation, no terbutaline was given to subjects with arrhythmias (n = 52). In the remaining participants, the software did not properly recognize the RI in 91 participants. Thus, the ΔRI could be evaluated in 793 subjects (86%). Common carotid IMT measurements had a CV of 7.2%.\textsuperscript{31}

**Follow-Up and Outcome Definitions**

The cohort was invited to a reexamination 1 month after their 75th birthday, which 827 individuals (81%) attended. The time between the examinations was 5.13 years (SD, 0.10 years). Participants were asked if they had been treated in hospital for a myocardial infarction or stroke. For those who had, the common computerized medical records for Uppsala County were evaluated by an experienced physician (L.L.) to validate the cases. Nonparticipants of the follow-up examination were contacted by telephone, and their medical records were screened to obtain their medical history at 75 years of age. By this action, only 2 participants were lost to follow-up. The reexamination was completed in September 2009.

The primary outcome, major adverse cardiovascular events (MACEs), was defined as all-cause mortality, hospital-treated myocardial infarction, or hospital-treated stroke during follow-up.

**Statistical Analysis**

Nonnormally distributed variables such as EDV, EIDV, serum triglycerides, fasting glucose, and C-reactive protein were log-transformed to achieve a normal distribution. Logistic regression analysis was used to investigate associations of the indexes of vascular function with risk of MACEs during the 5-year follow-up. Three hierarchical sets of models were investigated. The first models were adjusted for gender only. The second set of models was also adjusted for the Framingham risk score. The third set of models was adjusted for multiple risk factors: gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressures, high- and low-density lipoprotein cholesterol, serum triglycerides, smoking, antihypertensive treatment, antidiabetic treatment, statin usage, C-reactive protein, and carotid artery IMT. In all 3 sets of models, the indexes of vascular function were investigated both as a continuous variable (odds ratio [OR] per SD) and by quintiles (quintile 1 used as the reference group; quintile limits: 277%, 408%, 533.5%, and 719% for EDV; 2.3%, 3.3%, 5.5%, and 7.6% for FMD; and -43.6%, -35.4%, -28.2%, and -20.8% for ΔRI). We used generalized additive models (2 df) for graphic presentations of relations of vascular function indexes to risk of MACEs. Goodness of fit was evaluated by the Hosmer-Lemeshow test with 10 groups. The additional value of adding EDV to the Framingham risk score was evaluated by C statistics, likelihood ratio tests, and tests of reclassification (net reclassification improvement) and integrated discrimination improvement (IDI) according to the method proposed by Pencina et al.,\textsuperscript{34} as well as net clinical reclassification improvement. For the net reclassification improvement and net clinical reclassification improvement analyses, 3 risk groups were considered: <7% (low risk), 7% to 13% (intermediate risk), and ≥13% (high risk) risk of MACEs. To explore the cutoff for EDV yielding the best discrimination of risk of MACEs, we investigated all EDV cutoff points between percentiles 1 and 99 using step length--[(percentile 99--percentile 1)/98] defined binary variables (0 = below cutoff point; 1 = above or equal to cutoff point). For each cutoff point, IDI was calculated for comparison of discrimination improvement from...
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>921</td>
</tr>
<tr>
<td>Women, %</td>
<td>52.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169 (9.1)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77 (14)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>91 (11)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.9 (4.3)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>150 (23)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79 (10)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.4 (0.85)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5 (0.43)</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.3 (0.60)</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.3 (1.5)</td>
</tr>
<tr>
<td>Current smoking (prevalence), %</td>
<td>10</td>
</tr>
<tr>
<td>Framingham risk score</td>
<td>11.1 (3.3)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>3.1 (4.7)</td>
</tr>
<tr>
<td>Carotid artery IMT, mm</td>
<td>0.89 (0.16)</td>
</tr>
<tr>
<td>EDV, %</td>
<td>523 (298)</td>
</tr>
<tr>
<td>EIDV, %</td>
<td>369 (214)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>4.7 (3.6)</td>
</tr>
<tr>
<td>ΔRI, %</td>
<td>32 (14)</td>
</tr>
<tr>
<td>Angina pectoris, n (%)</td>
<td>50 (5.4)</td>
</tr>
<tr>
<td>CABG/PCI, n (%)</td>
<td>18 (2.0)</td>
</tr>
<tr>
<td>Congestive heart failure, n (%)</td>
<td>22 (2.4)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>72 (7.9)</td>
</tr>
<tr>
<td>Any regular drug use, n (%)</td>
<td>621 (67.4)</td>
</tr>
<tr>
<td>Any cardiovascular drug use, n (%)</td>
<td>371 (40.3)</td>
</tr>
<tr>
<td>Antihypertensive drug use, n (%)</td>
<td>259 (28.3)</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>100 (10.9)</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; IMT, intima-media thickness; EDV, endothelium-dependent vasodilation (invasive forearm technique); EIDV, endothelium-independent vasodilation (invasive forearm technique); FMD, flow-mediated dilatation; ΔRI, terebutaline-induced change in reflectance index by the pulse-wave–based technique; and CABG/PCI, coronary artery bypass graft/percutaneous coronary intervention. Data are mean (SD) when appropriate.

a model with only the Framingham risk score with a model that also contained the EDV variable. The optimal EDV cutoff point was defined as the cutoff point related to the maximum achieved IDI. The optimal cut points for EDV were obtained without validation in a separate cohort. Values of $P<0.05$ from 2-sided tests were considered statistically significant. The statistical software package Stata 10.0 (Stata Corp, College Station, TX) was used.

Results

Baseline characteristics of the sample are presented in Table 1.

Incidence of MACEs During Follow-Up

During follow-up, 42 participants died, 25 had a myocardial infarction, 32 had a stroke, and 2 suffered from both diseases, contributing to 101 instances of MACEs (risk=11.0%). Of the MACEs, 62 occurred in men and 39 in women.

Markers of EDV and Risk of MACEs

EDV was lower in subjects with a MACE over 5 years compared with those who did not experience any event (458% versus 531%; $P=0.0043$). No significant difference was seen for FMD or ΔRI (4.6% versus 4.7%, $P=0.68$; and −31% versus −32%, $P=0.67$).

No interactions between the 3 markers of EDV and gender regarding MACEs were found ($P=0.41$ for EDV, $P=0.81$ for FMD, and $P=0.78$ for ΔRI). Therefore, the further analyses were adjusted for, but not stratified by, gender.

The relation of EDV to 5-year risk of MACEs adjusting for gender was fairly linear in the −1 SD to 1 SD range (Figure 1A). This was not the case for FMD and ΔRI, which were not linearly related to risk of MACEs (Figure 1B and 1C).

In continuous models adjusted for gender, EDV, but not FMD or ΔRI, was a significant predictor of MACEs (Table 2). In addition, Framingham risk score (OR, 1.16 for 1-point increase; 95% confidence interval [CI], 1.09 to 1.24; $P<0.0001$) and C-reactive protein (OR, 1.04 for 1-unit increase; 95% CI, 1.01 to 1.08; $P=0.008$), but not IMT (OR, 2.77 for 1-mm increase; 95% CI, 0.76 to 10.0; $P=0.12$), were significantly related to MACEs. However, EDV was still significant when adjusted for gender and Framingham risk score and after adjustment for multiple traditional cardiovascular risk factors, carotid artery IMT, C-reactive protein, and cardiovascular medication use (Table 2). Of interest is the fact that EDV was the only variable among the 16 independent variables that was significantly associated with risk of MACEs (Table II in the online-only Data Supplement). Because statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers especially among the different drugs used might affect EDV, we investigated an additional model adjusting for use of these 3 drug classes; the OR for EDV remained virtually unchanged (OR, 0.74; 95% CI, 0.59 to 0.92; $P=0.007$). The other 2 markers of EDV were not significantly related to 5-year risk of MACEs after further adjustments (Table 2).

EDV and Risk of MACEs

No interaction between EIDV and gender on the 5-year risk of MACEs was observed ($P=0.84$). In continuous models, EIDV was weakly related to risk of MACEs with adjustment for gender (OR per 1 SD, 0.81; 95% CI, 0.66 to 1.00; $P=0.05$). After adjustment for Framingham risk score or multiple risk factors, EIDV was no longer significantly related to 5-year risk of MACEs.

EDV Improves Discrimination and Classification of Risk of MACEs

The addition of EDV to a model including the Framingham risk score as a predictor of MACEs increased the C-statistic significantly when adjusted for gender and Framingham risk score or after adjustment for multiple traditional cardiovascular risk factors, carotid artery IMT, C-reactive protein, and cardiovascular medication use (Table 2). Of interest is the fact that EDV was the only variable among the 16 independent variables that was significantly associated with risk of MACEs (Table II in the online-only Data Supplement). Because statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers especially among the different drugs used might affect EDV, we investigated an additional model adjusting for use of these 3 drug classes; the OR for EDV remained virtually unchanged (OR, 0.74; 95% CI, 0.59 to 0.92; $P=0.007$). The other 2 markers of EDV were not significantly related to 5-year risk of MACEs after further adjustments (Table 2).
in the intermediate-risk group were reclassified to the high-risk group. Furthermore, 24 of 341 MACE noncases in the intermediate-risk group were reclassified to the low-risk group (Table 3). The optimal cutoff level for EDV regarding discrimination of 5-year risk of MACEs was 580% (resulting in an IDI of 0.0013, \( P = 0.014 \); Figure 2), a value close to the mean value for EDV. The optimal cut points for EDV were obtained without validation in a separate cohort.

### Discussion

In the first community-based cohort study to date, EDV measured with the invasive forearm model was inversely associated with 5-year risk of a composite cardiovascular end point. Endothelium-dependent vasodilation improved risk discrimination beyond the established Framingham risk score. Although different measures of EDV previously have been related to risk of cardiovascular events,3,7,17–23 ours is the first study to compare the 3 most promising techniques in a large population-based study. Contrary to our hypothesis, we found that only vasodilation evoked by acetylcholine in forearm resistance vessels was a robust predictor of future MACEs during a 5-year follow-up in an elderly sample. This predictive power was seen independently of traditional cardiovascular risk factors, cardiovascular drug use, C-reactive protein, and carotid artery IMT. Furthermore, when added to the commonly used Framingham risk score, EDV significantly improved risk classification in the sample. The more commonly used FMD and the newer pulse-wave–based method to assess vasodilation were not significant predictors of future MACEs.

#### EDV With the Invasive Forearm Technique

Endothelium-dependent vasodilation previously has been shown to predict subsequent cardiovascular events in a sample of hypertensive persons7 and in samples of coronary heart disease patients.18,19 The number of patients was on the order of 200 in those studies. The present study is, to the best of our knowledge, the only large population-based study in which EDV has been evaluated longitudinally.

The predictive capacity of EDV was almost unaffected by adjustment for the Framingham risk score or a set of multiple risk factors. Table 2 shows the associations of EDV with major adverse cardiac events adjusted for gender and Framingham risk score and multiple individual risk factors. Logistic regression models of relations of 3 indexes of EDV and 1 index of EIDV as independent variables to risk of a combined end point of death, nonfatal myocardial infarction, and nonfatal stroke (n = 101) during 5 years of follow-up. The first set of models was adjusted for gender; the second set of models were adjusted for gender and the Framingham risk score; the third set of models were adjusted for multiple individual risk factors: gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressures, high- and low-density lipoprotein cholesterol, serum triglycerides, smoking, antihypertensive treatment, antidiabetic treatment, statin use, C-reactive protein, and common carotid artery intima-media thickness. In all 3 sets of models, the indexes of vascular function were investigated as a continuous variable (OR per SD).

### Table 2. Associations of Vascular Measures With Risk of Major Adverse Cardiac Events

<table>
<thead>
<tr>
<th></th>
<th>Models Adjusted for Gender</th>
<th>Models Adjusted for Gender and Framingham Risk Score</th>
<th>Multivariable-Adjusted Models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>EDV</td>
<td>0.74 (0.60–0.92)</td>
<td>0.007</td>
<td>0.78</td>
</tr>
<tr>
<td>FMD</td>
<td>0.95 (0.77–1.18)</td>
<td>0.65</td>
<td>0.99</td>
</tr>
<tr>
<td>ΔRI</td>
<td>0.98 (0.78–1.24)</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>EIDV</td>
<td>0.81 (0.66–1.00)</td>
<td>0.050</td>
<td>0.85</td>
</tr>
</tbody>
</table>

OR indicates odds ratio; CI, confidence interval; EDV, endothelium-dependent vasodilation; FMD, flow-mediated dilatation; ΔRI, terbutaline-induced change in reflectance index by the pulse-wave–based technique; and EIDV, endothelium-independent vasodilation.
cardiovascular risk factors, cardiovascular drug use, C-reactive protein, and carotid artery IMT. Thus, despite the fact that we have previously reported EDV to be related to the major risk factors included in the Framingham risk score, the metabolic syndrome, and C-reactive protein, EDV is apparently a powerful risk factor on its own.

Because we previously found the higher of the 2 acetylcholine doses evaluated to be more informative, EDV was calculated from that higher dose. Investigating the lower acetylcholine dose as a predictor of MACEs results in an OR per 1 SD of 0.87 (95% CI, 0.67 to 1.14). Thus, although a tendency to an association was also found with this lower dose, the higher dose of acetylcholine is needed to disclose a significant association with 5-year risk of MACEs.

Endothelium-Independent Vasodilation

The only measurement of EIDV performed in the present study was by the evaluation of sodium nitroprusside–induced vasodilation in the forearm resistance vessels. We have shown that sodium nitroprusside–induced vasodilation in the forearm resistance vessels is closely linked to glyceryl trinitrate–induced vasoreactivity in the brachial artery when measured by ultrasound. Endothelium-independent vasodilation in the present study was a borderline significant predictor of MACEs. Our results are in agreement with previous data on the predictive power of sodium nitroprusside–induced vasodilation in which either a borderline effect or no effect was found. Thus, although vasodilation in general might have some predictive power, it is obvious from these and other data that EDV is more important.

EDV Assessed by FMD

Two large population-based studies have demonstrated FMD to be related to risk of subsequent cardiovascular events, although the authors of one of the studies concluded that the additional contribution of FMD to the classic risk factors regarding risk prediction was very small. In the present study, we could not detect even a tendency of an association of FMD with 5-year risk of MACEs. There could be several reasons for this. First, FMD has a higher CV than EDV. This is likely not the sole explanation for the divergent findings because one of the previous studies reported a CV for FMD similar to that in the present study and still observed a highly significant relation of FMD to risk. Another, more likely, reason for the lack of an association in the present study is that FMD is heavily influenced by the deterioration in arterial compliance found also in healthy elderly subjects. Witte and coworkers demonstrated FMD to correlate with smoking and age in the expected way only in subjects with a normal arterial compli-

---

**Table 3. Reclassification of Risk of Cardiovascular Disease by Adding a Measure of Endothelium-Dependent Vasodilation to the Framingham Risk Score**

<table>
<thead>
<tr>
<th>Framingham Risk Score, %</th>
<th>Framingham Risk Score + EDV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;7%</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>13</td>
</tr>
<tr>
<td>7–13</td>
<td>36</td>
</tr>
<tr>
<td>≥13</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
</tr>
<tr>
<td>Noncases</td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>148</td>
</tr>
<tr>
<td>7–13</td>
<td>341</td>
</tr>
<tr>
<td>≥13</td>
<td>204</td>
</tr>
<tr>
<td>Total</td>
<td>693</td>
</tr>
</tbody>
</table>

Net reclassification improvement = 0.12, P = 0.01. Net clinical reclassification improvement in the intermediate group = 0.21, P = 0.03. EDV indicates endothelium-dependent vasodilation.

---

**Figure 2. Identification of the optimal cutoff value of dichotomized endothelium-dependent vasodilation for risk discrimination.** Relations of cutoff values for endothelium-dependent vasodilation (EDV; evaluated by the invasive forearm technique) to improvement in discrimination of 5-year risk of cardiovascular disease obtained when EDV dichotomized by that value is added to the Framingham risk score. Top line indicates a statistically significant integrated discrimination improvement (IDI).
ance, not in those with a poor compliance. We later reported that FMD was related to cardiovascular risk factors only in elderly subjects with a good arterial compliance, not in those with a poor compliance. Thus, the dependence on arterial compliance seen in the elderly might explain why FMD is not a good predictor of future MACEs in this elderly cohort.

**EDV Assessed by the Pulse-Wave–Based Method**

This is the first study to evaluate the ΔRI obtained by pulse-wave analysis as a predictor of subsequent cardiovascular events. The method previously has been shown to be related to EDV\(^\text{19}\) and to major cardiovascular risk factors,\(^\text{15}\) but in the present study, it was not a robust predictor of future cardiovascular events in the elderly. Thus, its clinical usefulness might be limited.

**Clinical Utility of the Invasive Forearm Technique**

Addition of EDV to the Framingham risk score improved the C statistic by 0.03 and IDI by 0.01 and improved classification of 5-year risk of MACEs. The inverse relation of EDV to risk of MACEs was fairly linear, and an optimal dichotomizing cutoff level for improved risk classification was close to the mean value for EDV, 2 characteristics supporting the use of EDV in future risk evaluation. The clinical usefulness of determinations of EDV has yet to be established, but its feasibility in population research is demonstrated here. The investigation takes 60 minutes and requires an arterial catheter. Our experience from almost 2000 of these procedures is good, with no other side effects except a bruise occurring in a minority of the subjects.

**Strengths and Limitations**

Study strengths include the large homogeneous sample, the standardized state-of-the-art vascular measurements, the very low attrition, and the chart review—based end-point adjudication. Some limitations are worth noting. The present sample consisted of white subjects 70 years of age, and generalizability to other ethnic and age groups is unknown. The present study had a moderate participation rate. However, an analysis of nonparticipants showed the present sample to be fairly representative of the total population in terms of most cardiovascular disorders and drug intake.\(^\text{15}\) A large number of participants in the present cohort regularly used cardiovascular drugs. Although no drugs were taken on the day of the investigation, differential effects of certain drugs on the risk factors and measures of endothelial vasodilatory function may exist that may have weakened the observed relations. For the sake of etiological understanding, the relations to a single disorder, such as myocardial infarction or stroke, should be studied. However, during the 5-year follow-up, too few events of those distinct disorders occurred in the present sample to make a robust risk assessment possible. Therefore, as in many clinical drug trials and in previous studies evaluating vasoreactivity as a risk factor, we used a composite MACE end point.

Still, the number of events is fairly low, so the analysis performed regarding the optimal cutoff value for EDV should be considered with caution. Because we cannot obtain the cause of death from the Swedish mortality registry for another 2 to 3 years, we used total mortality as a part of the MACE definition. This might have led to an underestimation of the association of EDV with cardiovascular disease.

In the present study, there is a lack of time-to-event data. Thus, logistic regression rather than Cox proportional hazard analysis was used.

**Conclusions**

EDV in resistance arteries, but not in the brachial conduit artery (FMD), was inversely associated with 5-year risk of a composite cardiovascular end point in this community-based cohort. EDV improved risk discrimination and classification beyond the established Framingham risk score. EDV assessment holds promise for population research use and possibly for clinical use if these results are replicated in future studies.

**Source of Funding**

This study was funded by the Swedish Research Council.

**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Impaired endothelial function has been implicated as a cause of cardiovascular disease. In 1016 participants (70 years of age) of the population-based prospective study of the vasculature in uppsala seniors (PIVUS) study (52% women), we measured endothelium-dependent vasodilation using the invasive forearm technique with acetylcholine given in the brachial artery (which evaluates vasoreactivity in resistance vessels), the brachial artery ultrasound technique with measurement of flow-mediated dilatation (which evaluates vasoreactivity in a larger conduit artery), and the pulse-wave analysis–based method with β-2-agonist terbutaline provocation (evaluating vasoreactivity in both large and small arteries). During 5 years of follow-up, 101 participants experienced a composite end point of myocardial infarction, stroke, or death. In logistic regression models adjusted for several established and novel cardiovascular disease risk factors and medications, endothelium-dependent vasodilation was associated with a risk of the end point (odds ratio, 0.72 per SD; 95% confidence interval, 0.56 to 0.93; P=0.01). Endothelial function by the other 2 methods was not related to risk of the end point. Addition of endothelium-dependent vasodilation to the Framingham risk score improved discrimination of risk of the end point. Thus, endothelium-dependent vasodilation in resistance arteries, but not in the brachial conduit artery (flow-mediated dilatation), was associated with 5-year risk of a composite end point of death, myocardial infarction, or stroke independently of major cardiovascular disease risk factors. The evaluation of endothelium-dependent vasodilation takes ~60 minutes to perform and might be used in the clinic in the future to improve risk classification.
Endothelial Function in Resistance and Conduit Arteries and 5-Year Risk of Cardiovascular Disease
Lars Lind, Lars Berglund, Anders Larsson and Johan Sundström

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SUPPLEMENTARY MATERIAL

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Lars Lind, MD PhD; Lars Berglund, PhD; Anders Larsson, MD PhD; Johan Sundström, MD PhD
Supplementary Table 1. Vascular tests

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 insertion. After evaluation of resting FBF, local intra-arterial drug-infusions were given during 5 minutes for each dose with a 20 minutes wash-out period between the drugs. The infused dosages were 25 and 50 ug/minute for Acetylcholine (Clinalfa, Switzerland) to evaluate EDV and 5 and 10 ug/minute for SNP (Nitropress, Abbot, UK) to evaluate EIDV. The drugs were given in a random order at a maximal rate of 1 ml/min. The dosages of Ach are slightly higher than those used by others. The reason for this is the use in elderly subjects being less responsive to Ach than younger subjects. We have before the study performed careful dose-response curves in elderly subjects with even higher doses showing that the highest dose used in the present study still is on the steep part of the dose-response curve. This was also the case for SNP, which is in the dose range used by most investigators (4-7).

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 ug/min of Acetylcholine minus resting
The brachial artery was assessed by external B-mode ultrasound imaging 2 – 3 cm above the elbow (Acuson, 7.0 MHz linear transducer, Acuson mountain view, California, USA) according to the recommendations of the International Brachial Artery Task Force (9). Depths and gains settings were optimised to identify the lumen to vessel wall interface. The subject rested in the supine position for at least 30 minutes 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 five minutes later, the artery was scanned continuously for 90 seconds and recorded on a super-VHS videotape for later analysis of the diameter. The diameter was manually measured at the peak of the R-wave at baseline and at 30, 60 and 90s following cuff deflation. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 sec following cuff release minus diameter at rest divided by the diameter at rest.

Due to the rather long time for the response to both nitroglycerine and terbutaline to disappear it was not for practical reasons possible to use both of these drugs during the investigation, which had to be taken place in the fasting state to avoid any influence of food. Since we previously have shown the the brachial artery diameter change to nitroglycerine is fairly well related to the forearm response to SNP (28), we chose to delete the nitroglycerine part.
Pulse wave analysis

A micromanometer tipped probe (Sphygmocor, Pulse Wave Medical Ltd, 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. The maximal systolic peak and the reflected waves were identified by the calculations of the first and second derivative of the pulse curve. After a baseline recording, terbutaline was subcutaneously administered (0.25 mg in the upper part of the arm) and a re-evaluation of the pulse wave was performed after 15 and 20 minutes. The maximal change occurring at either 15 or 20 min was used for calculations.

We have previously validated the relative height of the first diastolic reflected wave (the amplitude of the first systolic peak divided by the amplitude of the first diastolic reflected wave, here denoted reflection index, RI, see figure 1 in reference 15 for further details). The ΔRI denotes the relative change from baseline following terbutaline. Thus, a large reduction in RI indicates a large response.

The three different techniques to evaluate endothelium-dependent vasodilation were evaluated by three different persons unaware of the results of the other techniques or any clinical data. FMD and the pulse wave analysis were measured in the contra-lateral arm compared to the arterial cannulation. No participant had more than a 5 mmHg difference between the arms regarding SBP. The dominant and non-dominant arms were chosen in a random order.

Carotid artery intima-media thickness
The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA). The IMT was evaluated in the far wall in the common carotid artery (CCA) 1-2 cm proximal to the bulb.

The images were digitised and imported into the AMS (Artery Measurement Software) automated software (7). A maximal 10 mm segment with good image quality was chosen for IMT-analysis of the CCA. The programme automatically identifies the borders of the IMT of the far wall and the inner diameter of the vessel and calculates IMT from around 100 discrete measurements through the 10 mm long segment. This automated analysis could be manually corrected if not found appropriate at visual inspection. The given value for carotid artery IMT is the mean value from both sides.
Characteristics of non-attendants

As the participation rate in the baseline investigation of this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive non-participants. The prevalences of cardiovascular drug intake, history of myocardial infarction, coronary revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in the investigated sample, while the prevalences of diabetes, congestive heart failure and stroke tended to be higher among the non-participants (15).
**Supplementary table 2.** Logistic regression model with MACE as dependent variable and endothelium-dependent vasodilation (EDV, SD-transformed) together with 15 potential confounders as independent variables.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>OR</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>EDV</td>
<td>0.72</td>
<td>0.56-0.92</td>
<td>0.011</td>
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<tr>
<td>Gender</td>
<td>0.56</td>
<td>0.30-1.05</td>
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<td>Waist circumference</td>
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<td>0.83</td>
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<tr>
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<td>0.99-1.02</td>
<td>0.28</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
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<td>0.97-1.03</td>
<td>0.89</td>
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<tr>
<td>HDL</td>
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<tr>
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<td>0.99</td>
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<tr>
<td>Triglycerides</td>
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<td>Smoking</td>
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<td>0.81-3.29</td>
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<td>Antihypertensive treatment</td>
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<td>0.64-2.06</td>
<td>0.63</td>
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<tr>
<td>Antidiabetic treatment</td>
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<td>CRP</td>
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