

# Atherosclerosis and Lipoproteins

## A Comparison of Three Different Methods to Evaluate Endothelium-Dependent Vasodilation in the Elderly The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study

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**Background**—Three different techniques to evaluate endothelium-dependent vasodilation in the peripheral circulation have been described but not simultaneously tested in a large-scale population-based setting. This study aimed to evaluate the feasibility and usefulness of these techniques in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study.

**Methods and Results**—In the population-based PIVUS study (1016 subjects aged 70 years), the invasive forearm technique with acetylcholine given in the brachial artery (EDV), the brachial artery ultrasound technique with measurement of flow-mediated dilatation (FMD), and the pulse wave analysis method with  $\beta$ -2-agonist (terbutaline) provocation were successfully used in 87%, 97%, and 86% of the sample, respectively. The results of EDV and pulse wave analysis were interrelated ( $r=0.12$ ,  $P=0.0013$ ), but no relationships were found with FMD measurements. All 3 techniques were correlated to the Framingham risk score ( $r=0.10$  to  $0.12$ ,  $P=0.0007$  to  $0.001$ ). In multiple regression analysis, however, only EDV and FMD were independently associated with the Framingham score.

**Conclusions**— All 3 evaluated techniques were feasible to perform in a general elderly population. Both the invasive forearm technique and FMD were independently associated with increased coronary risk, suggesting that information on conduit artery and resistance artery endothelial function carry different, but important, information in the elderly. If the invasive technique cannot be used, the pulse wave based technique is an alternative. (*Arterioscler Thromb Vasc Biol.* 2005;25:2368-2375.)

**Key Words:** endothelium ■ vasodilation ■ cardiovascular ■ risk ■ elderly

Much attention has been paid to the evaluation of nitric oxide in the vasodilatory process in humans. Originally, endothelium-dependent vasodilation was evaluated as the vasomotor response to acetylcholine infused in a coronary artery.<sup>1</sup> This method seems to be valuable because impaired vasodilation could predict future cardiovascular events in patients with coronary disease,<sup>2</sup> but is restricted to subjects undergoing coronary angiography for clinical reasons.

The so-called invasive forearm technique uses acetylcholine to be infused in the brachial artery, and the increase in forearm blood flow is taken as a measure of endothelium-dependent vasodilation (here denoted EDV). This technique mainly evaluates endothelium-dependent vasodilation in forearm resistance arteries. A reduced EDV has been found in patients with coronary heart disease, hypertension, and hypercholesterolemia and in diabetes,<sup>3–6</sup> and also to predict future cardiovascular events.<sup>7</sup>

Another method to assess endothelium-dependent vasodilation is the ultrasound-based method evaluating flow-mediated vasodilation (FMD) in the brachial artery during

hyperemia.<sup>8,9</sup> This technique evaluates endothelium-dependent vasodilation in a conduit artery. Similar to EDV, it has been shown that FMD is attenuated in patients with coronary heart disease, and in those with major risk factors,<sup>10,11</sup> and can predict future cardiovascular events.<sup>12</sup>

Using aplanation tonometry of the radial artery to capture the peripheral pulse pressure waveform it was shown that reductions in the reflected waves induced by adrenergic  $\beta$ -2 receptor agonist stimulation could be blunted by the NO-blocker L-NMMA.<sup>13–15</sup> This technique mainly evaluates endothelium-dependent vasodilation in resistance arteries. A reduced response to  $\beta$ -2 agonists has been demonstrated in patients with hypercholesterolemia, CHD, and diabetes mellitus.<sup>13,14,16</sup>

When used in the same study, EDV and FMD in the brachial artery were not related in 2 different samples.<sup>17,18</sup> In the latter of these studies, the pulse wave based technique was found to be related to EDV, but not to FMD.<sup>18</sup> However, these 2 studies were rather small and not powered to detect also weak relationships.

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Several studies have used FMD in large-scale epidemiological research.<sup>19–24</sup> However, the other 2 mentioned techniques have not been used in this setting and very few studies have included more than 1 technique. Therefore, we conducted the Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study) with the primary aim to evaluate the power of 3 different tests of endothelium-dependent vasodilation in the peripheral circulation to predict future cardiovascular events in >1000 subjects aged 70 years living in the community of Uppsala, Sweden.

Because this primary aim will demand a long follow-up period, we here report 4 secondary aims of the study. First, to evaluate the feasibility to perform these 3 techniques in a large group of unselected elderly subjects. Second, to obtain reference values for the 3 methods in this age group. Third, to evaluate the relationships between the 3 different techniques in a large sample. Fourth, to investigate whether these 3 techniques were related to the Framingham risk score,<sup>25</sup> a widely used score to quantify risk for coronary heart disease.

## Materials and Methods

### Subjects

Eligible were all subjects aged 70 years living in the community of Uppsala, Sweden. 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 study was approved by the Ethics Committee of the University of Uppsala and the participants gave informed consent.

### Basic Investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication. All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After recordings of height, weight, 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 was carried out first followed by FMD and then the pulse wave based technique. At least 30 minutes passed between the different tests.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the noncannulated arm to nearest 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. Basic risk factors characteristics, medical history, and regular medication are given in Tables 1 and 2.

### Healthy Reference Groups

A group with no cardiovascular diagnosis or major risk factors was identified. The exclusion criteria were: History of any cardiovascular diagnosis or medication, obesity (body mass index [BMI] >30 kg/m<sup>2</sup>), hypertension (antihypertensive treatment or blood pressure >140/90 mm Hg), diabetes (antidiabetic treatment including diet or fasting blood glucose >6.1 mmol/L), hyperlipidemia (antihyperlipidemic treatment, LDL-cholesterol >3.5 mmol/L, or serum triglycerides >1.7 mmol/L), and current smoking. This group was denoted the PIVUS cardiovascular healthy reference group.

To give a descriptive young reference group, 10 young men and 10 young women (age 20 to 25 years) with the same exclusion criteria were investigated with an identical protocol. This group was denoted the young cardiovascular healthy reference group.

**TABLE 1. Basic Characteristics and Major Cardiovascular Risk Factors in the Total Sample and in a Cardiovascular Healthy Reference Group**

	Total Sample	PIVUS Healthy Reference Group	Young Healthy Reference Group
n	1016	131	20
Females, %	50.2	44.3	50
Height, cm	169 (9.1)	171 (10)	174 (8.7)
Weight, kg	77 (14)	72 (12)	67 (9.4)
Waist circumference, cm	91 (12)	86 (9.0)	77 (5.2)
BMI, kg/m <sup>2</sup>	27.0 (4.3)	24.6 (2.8)	22.2 (1.7)
Waist/hip ratio	0.90 (0.075)	0.88 (0.066)	0.81 (0.042)
SBP, mm Hg	150 (23)	125 (9.8)	113 (13)
DBP, mm Hg	79 (10)	72 (7.2)	68 (12)
Heart rate, beats/min	62 (8.7)	61 (9.0)	64 (13)
Serum cholesterol, mmol/L	5.4 (1.0)	5.2 (0.74)	3.8 (0.7)
LDL-cholesterol, mmol/L	3.3 (0.88)	3.1 (0.60)	2.1 (0.5)
HDL-cholesterol, mmol/L	1.5 (0.42)	1.6 (0.48)	1.4 (0.3)
Serum triglycerides, mmol/L	1.3 (0.60)	1.0 (0.42)	0.8 (0.3)
Fasting blood glucose, mmol/L	5.3 (1.6)	4.8 (0.5)	4.9 (0.4)
Current smoking, %	11	0	0

Means are given with SD in parenthesis. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Because the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive nonparticipants. 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, whereas the prevalences of diabetes, congestive heart failure and stroke tended to be higher among the nonparticipants (see Table 2).

### The Invasive Forearm Technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik). 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 5 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 minutes after cannula insertion. After evaluation of resting FBF, local intraarterial 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  $\mu$ g/min for Acetylcholine (Clin-Alpha) to evaluate EDV and 5 and 10  $\mu$ g/min for SNP (Nitropress, Abbot) to evaluate EIDV. The dosages of these drugs have been chosen to result in FBFs on the steep part of the dose-response curve without giving systemic effects. The drugs were given in a random order at a maximal rate of 1 mL/min.

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 of Acetylcholine minus resting FBF divided by resting FBF. EIDV was defined as FBF during infusion of 10  $\mu$ g/min of SNP minus resting FBF divided by resting FBF.

**TABLE 2. Self-Reported History of Cardiovascular (CV) Disorders and Regular Drug Intake Given in Percentage in the Investigated Sample and in 100 Nonattendees.**

	Total Investigated Sample	Not Attending
n	1016	100
Myocardial infarction	7.1	7.9
Stroke	3.7	6.7
Angina pectoris	8.1	13.8
CABG/PTCA	5.3	5.6
Congestive heart failure	3.8	6.9
Diabetes	8.7	16.9
Any regular drug	70	64
Any CV drug	45	52
Any antihypertensive medication	32	36
Beta-blockers	22	26
Calcium antagonists	11	12
Diuretics	13	19
ACE-inhibitors	8.5	11
Angiotensin II-blockers	8.3	9.1
GTN	3.0	3.4
Digoxin	2.1	9.2
Statins	15	17
Other antihyperlipidemic drugs	1.2	4.5
Insulin	1.8	1.1
Oral antidiabetic drugs	6.1	12
Warfarin	3.2	6.8
Aspirin/Clopidogrel	18	21
Other antiarrhythmic drugs	0.2	0

CABG/PTCA indicates coronary revascularization; GTN, any nitroglycerine preparation.

We did not use any distal cuff to exclude the hand circulation during measurements of EDV to avoid the discomfort and increase in sympathetic drive caused by this procedure. We have previously shown that almost identical results are obtained when FBF is measured with or without hand cuff in the resting state, or during infusion of SNP or another muscarinic receptor agonist, methacholine.<sup>26</sup> Own pilot studies showed that this is also valid during acetylcholine-mediated vasodilation, where no difference in FBF was seen and the correlation coefficient between cuff versus no cuff measurements was 0.92. We have previously shown the reproducibility (coefficient of variation, CV) for EDV and EIDV to be 8% to 10%.<sup>26</sup>

### The Brachial Artery Ultrasound Technique

The brachial artery was assessed by external B-mode ultrasound imaging 2 to 3 cm above the elbow (Acuson, 7.0 MHz linear transducer, Acuson Mountain View) according to the recommendations of the International Brachial Artery Task Force.<sup>9</sup> 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 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 mm Hg above systolic blood pressure. When the cuff was rapidly deflated 5 minutes later, the artery was scanned continuously for 90 seconds 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 sec after cuff deflation. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 sec after cuff release minus diameter at rest divided by the diameter at rest. The change in diameter is also given in absolute values. In accordance with the Framingham study,<sup>19</sup> FMD was also calculated for the 60 sec diameter value. We have previously shown the reproducibility (CV) to be 3% for baseline brachial artery diameter and 29% for FMD.<sup>17</sup>

### Pulse Wave Analysis

A micromanometer tipped probe (Sphygmocor, Pulse Wave Medical Ltd) 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  $\approx 10$  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 3 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. After a baseline recording, terbutaline was subcutaneously administered (0.25 mg in the upper part of the arm), and a reevaluation of the pulse wave was performed after 15 and 20 minutes. We had previously found that the maximal alterations in the pulse waveform occurred after 15 minutes in young healthy subjects,<sup>15</sup> but we performed a measurement also after 20 minutes in this sample of elderly subjects. Thus, the maximal change occurring at either 15 or 20 minutes was used for calculations.

In a previous studies, Hayward et al used the augmentation index derived from the first reflected wave (b/a; Figure I, available online at <http://atvb.ahajournals.org>) using the radial artery recording (here denoted AI-radial),<sup>14</sup> whereas Wilkinson et al used the augmentation index obtained after transformation of the radial pulse wave to the corresponding central pulse wave by use of a validated transfer function ((b-a)/a in Figure I; AI-aorta).<sup>13</sup> We have previously validated the relative height of diastolic reflected wave (d/a, Figure I; here denoted reflection index, RI).<sup>15</sup> Here, we report these 3 variables as changes relative changes from baseline after terbutaline. Thus, a large reduction of the pulse wave indices indicates a good response. We have previously shown the reproducibility (CV) for the change in RI and AI to be 9.4 and 16%, respectively.<sup>15</sup>

The 3 different techniques to evaluate endothelium-dependent vasodilation were evaluated by three different persons not aware 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 with the arterial cannulation. In no case a more than 5 mm Hg difference between the arms regarding SBP were seen excluding significant hemodynamic differences between the arms. The dominant and nondominant arms were chosen in a random order.

### Statistics

Nonnormally distributed variables were log-transformed to achieve a normal distribution. Relationships between pairs of variables were evaluated by Pearson correlation coefficient. Multiple regression analysis was applied to relate several independent variables to a dependent variable. Two-tailed significance values were given with  $P < 0.05$  regarded as significant. The statistical program package StatView (SAS Inc) was used.

## Results

### Feasibility to Perform the Methods

The EDV technique was not used in subjects on regular medication with 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 87% with a valuable test.

**TABLE 3. Means±SD or Median and 10th and 90th Percentile in Parenthesis for Vascular Variables in the Total Sample (n=1016), the PIVUS Cardiovascular Healthy Reference Group (n=131), and in the Young Cardiovascular Healthy Reference Group (n=20)**

Method	Variable	Total Sample	PIVUS Healthy Reference Group	Young Healthy Reference Group
Invasive	Basal FBF	4.0 (2.4–6.9)	3.8 (2.2–7.4)	4.2 (2.6–6.3)
Invasive	SNP, 5 µg/min	13.3 (8.1–21.9)	13.6 (8.5–22.9)	15.8 (6.3–30.9)
Invasive	SNP, 10 µg/min	17.3 (10.6–27.6)	17.4 (11.9–29.1)	23.3 (10.7–39.6)
Invasive	ACh, 25 µg/min	16.9 (8.1–29.1)	18.1 (7.1–31.5)	24.6 (6.4–37.1)
Invasive	ACh, 50 µg/min	23.4 (13.0–36.0)	26.4 (14.3–41.3)	29.0 (14.4–41.8)
Invasive	EDV, %	459 (199–909)	539 (224–1055)	614 (211–1248)
Invasive	EIDV, %	328 (149–629)	368 (158–740)	457 (151–885)
Ultrasound	Baseline diameter, mm	3.7±0.7	3.6±0.6	3.1±0.5
Ultrasound	Maximal diameter, mm	3.9±0.7	3.8±0.7	3.4±0.4
Ultrasound	Diameter change, mm	0.2 (0.0–0.3)	0.2 (0.0–0.4)	0.3 (0.1–0.5)
Ultrasound	FMD, %	4.4 (0.0–9.7)	5.4 (0.0–10.7)	9.4 (4.0–18.7)
PWA	Baseline AI-radial, %	89±18	84±18	45±15
PWA	Baseline AI-aorta, %	31±7	29±8	1±15
PWA	Baseline RI, %	45±8	45±7	46±9
PWA	AI-radial terbutaline, %	65±17	61±20	28±11
PWA	AI-aorta terbutaline, %	20±9	16±10	–13±12
PWA	RI terbutaline, %	31±8	30±8	28±7
PWA	% change AI-radial	–26±16	–28±20	–33±32
PWA	% change AI-aorta	–37±21	–47±43	a
PWA	% change RI	–31±14	–33±13	–40±14

Invasive indicates the invasive forearm technique; ultrasound, the brachial artery ultrasound technique; PWA, pulse wave analysis; FBF, forearm blood flow; SNP, sodium nitroprusside; Ach, Acetylcholine; EDV, endothelium-dependent vasodilation (invasive forearm technique); EIDV, endothelium-independent vasodilation (invasive forearm technique); The 5 first invasive variables are given in mL/min/100 mL tissue. FMD, flow mediated dilatation; AIx, augmentation index; RI, reflectance index.

a, cannot be given properly in relative values attributable to negative values following terbutaline.

The FMD technique was used in all subjects. In 27 subjects the artery could not be visualized in a proper way, resulting in valuable recordings in 97%.

After 3 cases of fainting after terbutaline injection in the first 300 subjects, occurring in subjects with frequent premature ventricular beats or atrial fibrillation, no terbutaline was given to subjects with these arrhythmias (n=52). In the remaining subjects, the software did not properly recognize AI-radial in 158 cases, AI-aortic in 129 cases, and RI in 91 subjects. Thus, evaluation of AI-radial could be performed in 79%, in 82% using AI-aortic, and in 86% of the sample by use of the RI.

### Distribution

The frequency distributions of the three tests are given in Figure II (available online at <http://atvb.ahajournals.org>). The change in RI (as well as changes in AI-radial and AI-aorta) was normally distributed, whereas the distributions of the 2 other tests were skewed. In the further analysis, these 2 latter tests were ln-transformed to achieve a normal distribution.

### Reference Values

In Table 3, the mean (or median) and variance for the different vascular variables are given in the total sample, the

PIVUS cardiovascular healthy reference group and the young healthy reference group. No significant differences between men and women were seen for the major variables (EDV, FMD and change in RI or AI).

### Relationships Between the Methods

EDV was related to the pulse wave based technique ( $r=-0.12$ ,  $P=0.0013$  for change in RI; Figure III, available online at <http://atvb.ahajournals.org>,  $r=-0.10$ ,  $P=0.0041$  for change in AI-aorta and  $r=-0.09$ ,  $P=0.013$  for change in AI-radial), but none of these techniques were related to FMD ( $r=-0.01$  to  $0.03$ ). Similar correlation coefficients were found when only the PIVUS cardiovascular healthy reference group was studied.

### Relationships With the Framingham Risk Score

All of the 3 techniques were correlated with the Framingham risk score, as could be seen in Figure IV (available online at <http://atvb.ahajournals.org>) ( $r=-0.12$ ,  $P=0.0007$  for EDV,  $r=-0.11$ ,  $P=0.0010$  for FMD and  $r=0.10$ ,  $P=0.0023$  for change in RI). No significant interactions were disclosed between gender and the 3 techniques in this respect.

When Framingham risk score was used as dependent variable in a multiple regression model with the 3 methods to

**TABLE 4. Univariate Relationships Between the Different Methods to Evaluate Endothelium-Dependent Vasodilation, Baseline Brachial Artery Diameter, and the Different Cardiovascular Risk Factors Included in the Framingham Risk Score Together With BMI**

	LDL-Cholesterol	HDL-Cholesterol	SBP	DBP	Fasting Glucose	BMI
EDV	0.04	0.13***	-0.07*	-0.15***	-0.06*	-0.24***
EIDV	0.08*	0.16***	-0.04	-0.05	-0.15***	-0.21***
Change in RI	-0.08*	-0.13***	0.06*	0.18***	0.04	0.13***
FMD	-0.01	-0.02	-0.09*	-0.07*	-0.03	0.01
Brachial artery diameter	0.12***	-0.26***	-0.02	0.16***	0.09**	0.17***

Pearson correlation coefficient is given.

EDV indicates endothelium-dependent vasodilation (invasive forearm technique); EIDV, endothelium-independent vasodilation (invasive forearm technique); RI, reflectance index at pulse wave analysis; FMD, the brachial artery ultrasound technique; SBP, systolic blood pressure; DBP, diastolic blood pressure.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

assess endothelial function as independent variables, only EDV ( $P=0.004$ ) and FMD ( $P=0.0003$ ) were significantly related to the Framingham risk score. However, if EDV was not used in the model, both FMD ( $P=0.0001$ ) and the change in RI ( $P=0.034$ ) were related to the Framingham risk score.

The use of other versions of measurements than those described above did not result in stronger correlations with the Framingham risk score ( $r=0.09$ ,  $P=0.0065$  for both AI-radial and AI-aorta,  $r=-0.11$ ,  $P=0.0001$  for FMD at 60 sec and  $r=-0.03$ ,  $P=0.35$  when FMD was measured in absolute values). Also EIDV evaluated by the invasive forearm technique was significantly correlated to the Framingham risk score ( $r=-0.11$ ,  $P=0.0012$ ).

The variables included in the Framingham risk score (LDL-cholesterol, HDL-cholesterol, blood pressure, and fasting blood glucose) were also analyzed separately in relation to the endothelial function measurements. The univariate correlation coefficients are given in Table 4. EDV was then found to be significantly related to all of these risk factors except LDL-cholesterol. The change in RI was found to be significantly related to all of the risk factors except fasting glucose, whereas FMD was related to blood pressure only. Adding a second grade order of these risk factors to detect nonlinear relationships did not substantially alter these relationships. None of the indices of endothelium-dependent vasodilation were significantly impaired in current smokers.

### EIDV Was Significantly Related to All of the Risk Factors Except Blood Pressure

In multiple regression analysis with the indices of endothelium-dependent vasodilation as dependent variables in multiple regression analysis and the above mentioned risk factors together with gender as independent variables, only HDL-cholesterol ( $P=0.0002$ ) and DBP ( $P=0.0003$ ) were significantly related to EDV. In a similar analysis with FMD as dependent variable, only SBP was independently related to FMD ( $P=0.014$ ), whereas DBP ( $P<0.0001$ ) was the only independent determinant of the change in RI. In a similar model, fasting blood glucose ( $P=0.0003$ ) and HDL-cholesterol ( $P=0.0001$ ) were independently related to EIDV. In univariate analysis, BMI was found to be significantly related to EDV, EIDV and the change in RI, but not to FMD (see

Table 4 for details). Baseline diameter of the brachial artery was significantly related to the major cardiovascular risk factors, except to SBP (see Table 4 for details).

## Discussion

The present study disclosed 4 major findings corresponding to the 4 aims of the study. First, it was feasible to perform 3 different tests of endothelium-dependent vasodilation, including the invasive forearm technique, in a large-scale population-based sample of elderly subjects. Second, reference values for these techniques are now available in subjects aged 70 years. Third, EDV and the pulse wave based technique are correlated, whereas FMD carries information not related to the other two tests. Fourth, all 3 techniques are related to coronary risk, as evaluated by the Framingham risk score. However, EDV and FMD were independently associated with coronary risk in multiple regression analysis, suggesting that information on conduit artery and resistance artery endothelial function carry different, but important, information in the elderly.

### Feasibility

Several studies have used FMD in the large-scale population setting and reported a high success rate,<sup>19–24</sup> in accordance with the present study. Although the other 2 techniques showed a lower feasibility than FMD in the present study (86% to 87%), the success rates in this age-group were similar to other methods widely used in epidemiological research, such as measurement of left ventricular mass with echocardiography.<sup>27</sup>

EDV should not be applied in subjects on anticoagulant agents because of risk of bleeding, but was safe in subjects on antiplatelet agents. We experienced fainting in some subjects with arrhythmias when giving terbutaline. However, many of the patients with arrhythmias have to be excluded from pulse wave analysis anyhow, because of a high variance in pulse wave data.

### Reference Values

In accordance with most investigators, we used the maximal diameter during the hyperaemic phase to calculate FMD. This might be why we report somewhat higher FMD compared

with the Framingham study, which used only measurements obtained at 60 sec of hyperemia.<sup>19</sup> However, also when we calculated FMD from the 60 sec recording only, our results were slightly higher (median 4.4 in the cardiovascular healthy).

We did not use a distal cuff to exclude the hand circulation when forearm blood flow was measured. Although the forearm circulation and the hand circulation might differ in some aspects, this fact does in practice not influence the evaluation of EDV and EIDV, as we have shown that EDV and EIDV were not altered when EDV and EIDV were measured both with and without a wrist cuff.<sup>26</sup>

Although the study was not designed to evaluate differences between young and elderly healthy individuals and therefore no statistical tests were performed, higher numerical responses were seen in the younger subjects compared with the healthy elderly, in accordance with previous studies.<sup>28,29</sup>

### Relationships Between the Methods

We have previously presented similar data as found in the present study, namely that EDV and the change in RI are related, whereas no correlation is found with FMD.<sup>17,18</sup> Taken together, these observations strongly suggests that endothelium-dependent vasodilation in resistance arteries (the invasive forearm technique and the pulse wave based technique) and in a conduit artery (the FMD method) differ substantially and might be governed by different mechanisms. Another difference between the technologies is that FMD uses shear stress as the stimuli for vasodilation, whereas in the other 2 cases vasodilation is evoked pharmacologically by receptor agonists.

Also other investigators have found EDV and the pulse wave-based technique to be related,<sup>19</sup> whereas a small study (n=16) reported a close correlation between EDV and FMD. In that study the majority of subjects were hypertensive, so it might be that particular disease state that caused the high correlation.<sup>30</sup> A recent study found the effects of acetylcholine and the  $\beta$ -2 agonist salbutamol to be similar when given locally in the coronary arteries.<sup>31</sup>

### Relationships With the Framingham Risk Score

As in accordance with the Framingham study,<sup>19</sup> we also found a relation between FMD and coronary risk. The correlation coefficient in the present study was less powerful compared with the one found in the Framingham study, but was higher compared with the one found in the FATE study.<sup>21</sup> The participants in the Framingham study were on the average 61 years old, and it has been argued that FMD loose in predictive power with ageing.<sup>34</sup> Using the fixed 60 sec measurement for FMD or the change in diameter in absolute numbers did not improve the relation to coronary risk. When the risk factors were analyzed on an individual basis, blood pressure was the main determinant of FMD.

In previous studies relating EDV to the Framingham risk score in middle-aged subjects,<sup>32,33</sup> acetylcholine-mediated vasodilation was also found to be related to the Framingham risk score with a similar magnitude as found in the present study.

The most interesting finding in the present study is that EDV and FMD were independently related to coronary risk, suggesting that both methods carry unique and important information. The invasive forearm technique evaluates change in blood flow mainly in resistance vessels, whereas FMD evaluates diameter change in a conductance artery. The change in RI was less valuable in this respect, but if EDV measurements not could be conducted also endothelium-dependent vasodilation evaluated by the pulse wave-based technique added significant information to FMD regarding coronary risk.

Although it was not included in the Framingham risk score it was of interest to analyze also the relationship between endothelium-dependent vasodilation and BMI, as the Framingham study reported a negative relationship between FMD and BMI<sup>19</sup> and The Cardiovascular Risk in Young Finns Study on the contrary reported a positive association.<sup>20</sup> In the present study no relationship between BMI and FMD could be detected even when nonlinear relationships were considered, but obesity was related to an impaired endothelium-dependent vasodilation when evaluated by both EDV and the change in RI (Table 4).

Also EIDV evaluated by the invasive forearm technique was significantly related to the Framingham risk score. Whereas many investigators not found EIDV to be related to coronary heart disease or different cardiovascular risk factors,<sup>4,5</sup> others have.<sup>35,36</sup> EIDV is regarded to mainly evaluate structural changes in the vascular wall, such as increased vascular stiffness attributable to the loss of elastin fibers and an increase in collagen fibers and smooth muscle cells in the media layer. These alterations in the arterial structure are seen with increasing frequency with age, and it seems likely that these alterations in vascular morphology would be related to major cardiovascular risk factors in elderly subjects. In the present study both EDV and EIDV were numerically lower in the healthy elderly compared with the healthy young controls, suggesting that also a reduced EIDV is a part of the ageing process even in the apparently healthy elderly.

Also the baseline brachial artery diameter was found to be related to the major cardiovascular risk factors in accordance with the findings in the Framingham study.<sup>19</sup> The reason for this is not clear. Conduit arteries are known to increase in diameter with age,<sup>19</sup> and the baseline brachial artery diameter was numerically higher in the healthy elderly compared with the healthy young controls, suggesting that an increase in conduit artery diameter is a part of the ageing process even in the apparently healthy elderly, in analogy with EIDV.

### Comments to the Pulse Wave-Based Technique

In the present study, we mainly present pulse wave data as change in RI. This was because changes in AI-radial and AI-aorta were slightly less feasible to obtain and not more closely related to coronary risk than RI. It should however be emphasized that the differences between the three ways to evaluate endothelium-dependent vasodilation with the pulse wave based-technique were small and that all three variables could be used.

### Limitation of the Study

The present sample is limited to Whites aged 70 years. So, caution should be made to draw conclusions to other ethnic and age groups.

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 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, as we would have had to give GNT before terbutaline and would have had to wait for the withdrawal of the GNT effect. Furthermore, we have previously shown that EIDV evaluated by SNP infusion in the brachial artery and GNT provoked change in brachial artery diameter are closely related,<sup>17</sup> so additional measurements of EIDV would probably not add substantial information to the study.

A large number of subjects in the present cohort were on regular cardiovascular medications. Although no drugs were taken at the day of the investigation, differential effects of certain drugs on the risk factors and on the measures of endothelial vasodilatory function may exist that could have weakened these relationships.

### Conclusion

All 3 evaluated techniques to evaluate endothelium-dependent vasodilation were feasible to perform in a general elderly population. Both the EDV and FMD were independently associated with increased coronary risk, suggesting that information on conduit artery and resistance artery endothelial function carry different, but important, information in the elderly. However, if EDV cannot be used, the pulse wave based technique is an alternative.

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