Noninvasive Vascular Function Measurement in the Community
Cross-Sectional Relations and Comparison of Methods

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Background—Several methods of noninvasive vascular function testing have been suggested for cardiovascular risk screening in the community. A direct comparison of the different methods and their relation to classical cardiovascular risk factors in a large cohort is missing.

Methods and Results—In 5000 individuals (mean age, 55.5 ± 10.9 years; age range, 35 to 74 years; women, 49.2%) of the population-based Gutenberg Heart Study, we performed simultaneous measurement of flow-mediated dilation (FMD) and peripheral arterial volume pulse determined by infrared photo (reflection index) and pneumatic plethysmography (PAT) and explored their associations. All function measures were recorded at baseline and after reactive hyperemia induced by 5-minute brachial artery occlusion. Correlations between different measures of vascular function were statistically significant but moderate. The strongest association for hyperemic response variables was observed for PAT ratio and FMD (Spearman \( r = 0.17 \); age- and sex-adjusted partial correlation, 0.068). Classical risk factors explained between 15.8% (baseline reflection index) and 58.4% (brachial artery diameter) of the baseline values but only accounted for 3.2% (reflection index), 15.4% (FMD), and 13.9% (PAT ratio) of the variability of reflective hyperemic response. Regression models varied in their relations to classical risk factors for the individual vascular function measures. Consistently associated with different vascular function methods were age, sex, body mass index, and indicators of hypertension. Peripheral tonometry also showed a relation to fasting glucose concentrations.

Conclusions—Noninvasive measures of conduit artery and peripheral arterial function are modestly correlated, differ in their relation to classical cardiovascular risk factors, and may thus reflect different pathologies. (Circ Cardiovasc Imaging. 2011;4:371-380.)

Key Words: manometry ■ cross-sectional studies ■ vascular endothelium-dependent relaxation ■ endothelium, vascular

The endothelium is responsible for the fine tuning of vascular homeostasis and mirrors current vascular function. Endothelial dysfunction in the coronary arteries is directly related to the incidence of coronary events. Noninvasive vascular function measured by flow-mediated dilation (FMD) or peripheral arterial tonometry (PAT) is positively correlated with impaired coronary endothelial function and has become an attractive tool to assess vascular stress burden. It has been suggested that endothelial function is an integration of risk factors affecting vascular function and may provide a picture of the current vascular status and the risk of future cardiovascular events. Methods for determination of noninvasive endothelial function are the assessment of arterial morphology and function by FMD of conductance arteries and PAT by registering finger pulse volume and infrared light transmission plethysmography. What all 3 function measures have in common is that they are, at least in part, affected by the bioavailability of nitric oxide, the molecule central to vascular homeostasis and regulation of endothelial reactivity. FMD reflects large artery reactivity; finger plethysmography helps to assess microvascular function in the terminal vascular bed. Whereas clinical correlates of FMD and PAT in the community have been reported in US cohorts, we present data in a large, contemporary cohort with simultaneous determination of vascular function, including en-
dothelial reactivity, with 3 different, noninvasively applicable methods.

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Methods

Participants

Vascular function was determined in 5000 individuals of the Gutenberg Heart Study, a cohort conceptualized for examination of cardiovascular disease. From 2007 on, residents of the city of Mainz and the county Mainz/Bingen aged 34 to 74 years were selected within age strata from a random sample through the registration office. Details on enrollment, risk factor assessment, and laboratory methods are provided in the online-only Data Supplement. The project was approved by the local Ethics Committee. All participants provided written, informed consent. All authors have read and agreed to the manuscript as written.

Flow-Mediated Dilation

FMD was measured following guidelines using a 5-minute upper-arm occlusion and the brachial artery diameter measured in resting conditions and after induction of local reactive hyperemia. In brief, measurements were performed in dark, air-conditioned rooms after at least 5 minutes of rest and before blood draw by technicians who had performed >250 vascular function studies before the beginning of study enrollment. Two-dimensional high-resolution ultrasonic imaging of the right brachial artery was performed on a Philips HD11XE CV ultrasound machine (Best, The Netherlands) using a linear array broadband probe, 1L2–5 (38 mm). Baseline loops and loops recorded 60 seconds after cuff release were saved digitally, and subsequently, artery diameters were analyzed semiautomatically 3 times on an off-line reading station with commercially available software (Brachial Analyzer version 5.0, Medical Imaging Applications LLC; Iowa City, IA). Performance according to standard operating procedures was achieved in 97.6% of examinations. Six individuals refused to take part in the vascular function measurement. We further excluded studies based on bad image quality and technically inadequate studies (insufficient flow occlusion, occlusion for <5 minutes, incomplete data acquisition) (n=168). Reproducibility of the measurements under standardized conditions is good.

Peripheral Arterial Tonometry

Pneumatic Plethysmography

Pneumatic pulse amplitude was recorded by the Endo-PAT2000 device (Itamar Medical; Caesarea, Israel) under comparable conditions as reported in recent investigations. Pulse amplitude was registered electronically in both index fingers, with the left index finger serving as the control finger. The results were automatically derived by a computerized algorithm. Quality criteria and reasons to exclude PAT studies (n=189) were noisy signals (region of interest <80% of valid signals), occlusion duration >5.5 or <4.5 minutes, and breakthrough of the arterial pulse curve during upper-arm occlusion. High-quality data were available in 96.2% of studies. Because of data transfer problems, we were not able to read and evaluate the first 336 data sets.

Infrared Plethysmography

The digital volume pulse was continuously obtained by measuring the transmission of infrared light through the finger pulp of the right ring (or fourth) finger with a PulseTrace 2000 device (Micro Medical Limited; Rochester, UK). The waveform was automatically analyzed to identify the notch or inflection point (the point at which the first derivative of the waveform reaches a second maximum after the first peak of the waveform) corresponding to the component of pulse contour reflected from the lower body. The reflection index, a measure of vascular tone, was automatically calculated as the relative amplitude of the forward wave and reflected wave component. In stiff arteries, the 2 wave components can no longer be discriminated, and the study cannot be evaluated; we excluded individuals with impaired signal quality (n=26).

All 3 vascular function measurements were performed simultaneously in 1 examination by technicians trained according to protocol and with continuous quality control. Reproducibility data are provided in online-only Data Supplement Table 1. As known from the literature, reproducibility of FMD and PAT under standardized conditions is good, whereas index variability was high. Finger girth also was recorded.

Statistical Analysis

We focused on major response variables and examined the following indicators of vascular function: brachial artery diameter at baseline (rest) and 60 seconds after cuff release (hyperemia), FMD percent, pulse amplitude in resting conditions, PAT ratio, baseline reflection index, and reflection index at hyperemia. No standardized presentation of hyperemic response reflection index has been reported. Because the hyperemic reflection index compared to its baseline value centered on 0, we decided to show the difference across the 2 measurements. Other statistical transformations would have resulted in extreme distributions. Skewed variables were logarithmically transformed to achieve near-normal distribution.

We defined a reference group of individuals with no manifest cardiovascular disease (history of myocardial infarction, coronary heart disease, heart failure, and stroke) and no known classical cardiovascular risk factors. Individuals (n=5) who did not report classical risk factors but had manifest cardiovascular disease were analyzed in the group with classical risk factors. We calculated Spearman correlation coefficients for the vascular function measures adjusted for age and sex. Vascular function variables were plotted by 10-year age increments. Linear regression models were run for classical cardiovascular risk factors in relation to selected vascular function measures. To enhance comparability across different vascular function measures, response variables were standardized (X-mean/SD) to achieve a mean of 0 and SD of 1. We determined the association between sex-specific vascular function variables and quintiles of the European Society of Cardiology score.

Individuals outside the validated risk score range were grouped into the lowest age group if aged <45 years or highest age group if aged >65 years. P<0.05 was chosen as the statistical significance threshold, although analyses have to be considered exploratory, and results need to be replicated in independent cohorts. In a secondary analysis, we assessed glucose variables (normal fasting glucose, impaired fasting glucose [100 to 125 mg/dL], or diabetes) in relation to vascular function measures. Data were analyzed using R version 2.10.1 software (http://www.R-project.org).

Results

The characteristics of the total sample are broken down by sex and for the reference group as shown in Table 1. The mean age was 55.5±10.9 years; 49.2% of the cohort were women. In contrast, the reference sample showed a mean age of 50.2±10.4 years, with 60% women. Overall, men tended to have a higher cardiovascular risk factor burden. Women had lower resting brachial artery diameter, reflection index, and pulse amplitude but a stronger response to hyperemia as measured by both FMD and PAT (Table 2).

Hyperemic reflection index did not show a clear direction toward an increase or decrease in the overall sample (mean±SD difference, −2.24±13.34). In online-only Data Supplement Table 2, the characteristics of the sample are provided by increase versus decrease of hyperemic reflection index. In general, individuals with a decrease in reflection index were younger and showed a more-favorable risk factor distribution, better vascular function (PAT ratio and FMD), and lower risk score values.

Moderate partial correlation coefficients were observed for baseline measures; absolute values of coefficients between hyperemic response variables were <0.1 (Table 3). Plots for vascular function measures by 10-year age increments re-
Table 1. Demographics and Classical Risk Factors of the Overall Cohort and the Reference Group

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>Total Sample</th>
<th>Reference Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=2540)</td>
<td>(n=2460)</td>
<td>(N=5000)</td>
<td>(n=1037)</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.0±10.9</td>
<td>55.0±11.0</td>
<td>55.5±10.9</td>
<td>50.2±10.4</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>136±16</td>
<td>131±18</td>
<td>134±18</td>
<td>122±12</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80±9</td>
<td>78±9</td>
<td>79±9</td>
<td>74±6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>64±11</td>
<td>65±10</td>
<td>64±10</td>
<td>62±9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.77±0.07</td>
<td>1.64±0.07</td>
<td>1.70±0.09</td>
<td>1.71±0.09</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.7±4.1</td>
<td>26.7±5.4</td>
<td>27.2±4.8</td>
<td>24.4±2.8</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>4.6±1.3</td>
<td>3.8±1.0</td>
<td>4.2±1.2</td>
<td>3.5±0.8</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>96.4±18.1</td>
<td>92.0±16.5</td>
<td>94.3±17.5</td>
<td>88.5±7.3</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.6 (0.5, 3.0)</td>
<td>1.7 (1.0, 3.7)</td>
<td>1.7 (0.5, 3.3)</td>
<td>1.20 (0.50, 2.10)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>247 (9.7)</td>
<td>127 (5.2)</td>
<td>374 (7.5)</td>
<td>...</td>
</tr>
<tr>
<td>Current smoking</td>
<td>527 (20.8)</td>
<td>432 (17.6)</td>
<td>959 (19.2)</td>
<td>...</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1426 (56.1)</td>
<td>1138 (46.3)</td>
<td>2564 (51.3)</td>
<td>...</td>
</tr>
<tr>
<td>Hypertension treatment</td>
<td>755 (29.7)</td>
<td>677 (27.5)</td>
<td>1432 (28.7)</td>
<td>...</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>923 (36.4)</td>
<td>539 (21.9)</td>
<td>1462 (29.3)</td>
<td>...</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease</td>
<td>276 (10.9)</td>
<td>131 (5.3)</td>
<td>407 (8.1)</td>
<td>...</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, median (25th percentile, 75th percentile), or n (%). BMI indicates body mass index; HDL, high-density lipoprotein cholesterol.

Revealed an increase in baseline values (Figure 1) and showed a decrease for hyperemic response for PAT ratio in men and FMD in women. Reflection index difference increased in both sexes in the total sample. Trends were comparable in the reference group (online-only Data Supplement Figure 1).

Correlation With Cardiovascular Risk Factors

Linear regression analysis showed that most cardiovascular risk factors, including C-reactive protein as a prognostic inflammatory biomarker, were correlated with vascular function measures (Tables 4 to 6).

Table 2. Vascular Function Measures in the Overall Cohort and the Reference Group

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Median</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>(25th, 75th Percentile)</td>
<td></td>
<td>(25th, 75th Percentile)</td>
</tr>
<tr>
<td>Overall sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basilar arterial diameter, mm</td>
<td>4.89±0.59</td>
<td>4.92 (4.52, 5.29)</td>
<td>3.74±0.59</td>
</tr>
<tr>
<td>FMD, %</td>
<td>6.53±3.73</td>
<td>6.05 (3.87, 8.71)</td>
<td>9.69±5.39</td>
</tr>
<tr>
<td>Basilar pulse amplitude</td>
<td>6.42±0.74</td>
<td>6.54 (5.97, 6.98)</td>
<td>5.67±0.90</td>
</tr>
<tr>
<td>PAT ratio</td>
<td>0.44±0.40</td>
<td>0.39 (0.15, 0.72)</td>
<td>0.67±0.46</td>
</tr>
<tr>
<td>Basilar RI</td>
<td>72.57±13.40</td>
<td>75.0 (66.0, 82.0)</td>
<td>61.35±14.38</td>
</tr>
<tr>
<td>Hyperemic RI</td>
<td>71.99±13.17</td>
<td>75.0 (67.0, 81.0)</td>
<td>57.38±15.81</td>
</tr>
<tr>
<td>RI difference</td>
<td>-0.65±11.74</td>
<td>-1.0 (-5.00, 3.0)</td>
<td>-3.93±14.67</td>
</tr>
<tr>
<td>Reference sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basilar arterial diameter, mm</td>
<td>4.73±0.60</td>
<td>4.76 (4.37, 5.14)</td>
<td>3.53±0.55</td>
</tr>
<tr>
<td>FMD, %</td>
<td>6.65±3.84</td>
<td>6.29 (3.93, 8.72)</td>
<td>11.30±5.70</td>
</tr>
<tr>
<td>Basilar pulse amplitude</td>
<td>6.28±0.79</td>
<td>6.40 (5.78, 6.86)</td>
<td>5.39±0.90</td>
</tr>
<tr>
<td>PAT ratio</td>
<td>0.47±0.38</td>
<td>0.45 (0.17, 0.75)</td>
<td>0.71±0.46</td>
</tr>
<tr>
<td>Basilar RI</td>
<td>72.6±13.8</td>
<td>75.0 (66.0, 82.0)</td>
<td>60.3±13.6</td>
</tr>
<tr>
<td>Hyperemic RI</td>
<td>71.8±12.7</td>
<td>74.0 (67.0, 81.0)</td>
<td>54.6±14.3</td>
</tr>
<tr>
<td>RI difference</td>
<td>-0.83±11.4</td>
<td>-1.0 (-6.0, 4.0)</td>
<td>-5.83±12.70</td>
</tr>
</tbody>
</table>

FMD indicates flow-mediated dilation; PAT, peripheral arterial tonometry; RI, reflection index.
Flow-Mediated Dilation
Whereas 58.4% of variability of baseline brachial artery diameter was explained by clinical correlates, only 15.4% of the variability in hyperemic response was accounted for. Strongest associations were observed for sex, hypertension, and antihypertensive medication (Table 4). For FMD, body mass index (BMI), diabetes, and dyslipidemia showed high β-estimates besides sex and hypertension variables.

Peripheral Arterial Tonometry
Clinical correlates explained between 3.0% (reflection index differences) and 29.3% (baseline volume pulse) of the variability of PAT measures. Strongest associations with baseline pulse amplitude were observed for age, sex, current smoking, total and high-density lipoprotein cholesterol, diabetes, dyslipidemia, and variables reflecting hypertension (Tables 5 and 6). In general, relations with hyperemic response variables were weaker. Significant associations for both tonometry methods were seen for age, sex, BMI, fasting blood glucose, dyslipidemia, and hypertension treatment. Overall, the strongest predictors of vascular function were age and sex. Consistently associated across different methods of vascular function measurement were age, BMI, and blood pressure variables. For peripheral tonometry, fasting blood glucose also revealed comparatively strong associations for baseline variables as well as for the hyperemic response. Online-only Data Supplement Figure 2 summarizes graphically the significant correlations between cardiovascular risk factors and vascular function measures. Values remained similar after adjustment for classical cardiovascular risk factors (data not shown). Online-only Data Supplement Table 3 provides the distribution of vascular function measures in individuals without cardiovascular risk factors as a healthy reference group.

Correlation With Cardiovascular Risk Scores
Figure 2 shows mean values of vascular function measures by quartiles of risk assessed by the European Society of Cardiology risk score. Baseline variables increased over quartiles of risk in both sexes, with lower values in women. Strongest changes were observed for baseline brachial artery diameter and only small increases for reflection index. For hyperemic response, a decrease in vascular function with increasing risk could be demonstrated for FMD and PAT ratio in women. Trends were less striking in men and for reflection index difference.

BMI, Finger Girth, and Vascular Function
BMI was moderately correlated with finger girth (Spearman r=0.29). The correlations of BMI with vascular function measures were strongest for baseline brachial artery diameter (r=0.34) and pulse amplitude (r=0.36) (online-only Data Supplement Table 4). Partial correlation coefficients accounting for age, sex, and finger girth only mildly attenuated correlations with vascular function measures.

Glucose Metabolism and Vascular Function
Impaired fasting glucose and diabetes were significantly associated with adverse vascular function (online-only Data Supplement Figure 3). Strongest vascular function impairment was observed in individuals with manifest diabetes.

Discussion
The present data reflect the simultaneous, high-quality acquisition of 3 noninvasive vascular function measures in a large, unselected, population-based cohort. Strongest clinical correlates of all vascular function were age and sex followed by BMI and indicators of high blood pressure, showing the importance of age- and sex-specific normal values. Whereas baseline parameters were correlated with a broad spectrum of cardiovascular risk factors, the correlation with hyperemic response variables was weaker, and the overall variability explained by the risk factors was lower.

Hyperemic reflection index changes in response to hyperemia on average were almost 0. Based on experimental data, we had expected a decrease in the reflection index during postischemic hyperemia due to higher nitric oxide availability. Compared with individuals with a higher reflection index after hyperemia, those with a decrease in reflection index were younger and had lower risk factor burden. Whether these subtle differences in reflection index may help to identify individuals at increased risk for the development of cardiovascular disease needs to be investigated.

Sound experimental evidence links hypertension with a reduced bioavailability of nitric oxide due to impaired synthesis and enhanced degradation by various mechanisms. BMI also has repeatedly been related to impaired peripheral vascular function. The mechanisms are less well understood. Insulin resistance, systemic inflammation, and oxidative

| Table 3. Partial Correlations of Vascular Function Measures Adjusted for Age and Sex |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | Baseline Pulse  | PAT Ratio       | Baseline Brachial | FMD              | RI              | RI Difference   |
|                                  | Amplitude       |                 | Artery Diameter  |                 |                 |                 |
| Baseline pulse amplitude         | ...             | -0.67           | 0.23             | -0.079           | 0.26            | 0.0035          |
| PAT ratio                        | <0.0001         | ...             | -0.16            | 0.068            | -0.19           | -0.027          |
| Baseline brachial artery diameter| <0.0001         | <0.0001         | ...              | -0.37            | 0.045           | 0.0082          |
| FMD                              | <0.0001         | <0.0001         | <0.0001          | ...              | -0.040          | 0.0043          |
| RI                               | <0.0001         | <0.0001         | 0.0019           | 0.0060           | ...             | -0.35           |
| RI difference                    | 0.82            | 0.074           | 0.58             | 0.77             | <0.0001         | ...             |

Data are presented as Spearman rank correlation coefficients. Abbreviations as in Table 2.
Figure 1. Vascular function measures by 10-year increments in age in the overall sample. A, FMD. B, Volume plethysmographic PAT. C, Reflection index. *P* values were derived by Jonckheere-Terpstra trend test. FMD indicates flow-mediated dilation; PAT, peripheral arterial tonometry.
stress seem to be main pathways that impair endothelial homeostasis and need further investigation. Associations with other classical cardiovascular risk factors differed not only among the 3 methods, but also for the relation with baseline variables and hyperemic response. We observed a stronger correlation of classical cardiovascular risk factors with baseline values, such as arterial diameter and baseline pulse amplitude compared to dynamic changes after reactive hyperemia. Vascular function measures as determined in the present study represent indicators of long-term

### Table 4. Linear Regression Models for Classical Risk Factors in Relation to FMD Adjusted for Age and Sex

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Brachial Diameter</th>
<th>FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.25</td>
<td>0.23 to 0.26</td>
</tr>
<tr>
<td>Female sex</td>
<td>-1.4</td>
<td>-1.4 to -1.3</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>0.20</td>
<td>0.18 to 0.21</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-0.067</td>
<td>-0.12 to -0.018</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>0.066</td>
<td>0.045 to 0.087</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>0.059</td>
<td>0.040 to 0.078</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>0.015</td>
<td>-0.0045 to 0.034</td>
</tr>
<tr>
<td>Height, m</td>
<td>0.082</td>
<td>0.054 to 0.11</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>0.053</td>
<td>0.032 to 0.073</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.046</td>
<td>0.026 to 0.066</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>0.053</td>
<td>0.033 to 0.073</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>0.042</td>
<td>0.023 to 0.062</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.089</td>
<td>0.013 to 0.16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.16</td>
<td>0.12 to 0.20</td>
</tr>
<tr>
<td>Hypertension treatment</td>
<td>0.17</td>
<td>0.12 to 0.21</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.045</td>
<td>0.0029 to 0.088</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease</td>
<td>-0.036</td>
<td>-0.11 to -0.037</td>
</tr>
</tbody>
</table>

Model $R^2$ for baseline diameter, 58.4%; FMD, 15.4%. Regression coefficients are for standardized (mean, 0; SD, 1) continuous variables. Age was adjusted for sex; sex was adjusted for age. Triglyceride and C-reactive protein levels were logarithmically transformed for analyses. Abbreviations as in Tables 1 and 2.

### Table 5. Linear Regression Models for Classical Risk Factors in Relation to PAT (Plethysmographic Volume Pulse) Adjusted for Age and Sex

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Pulse Amplitude</th>
<th>PAT Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.21</td>
<td>0.18 to 0.24</td>
</tr>
<tr>
<td>Female sex</td>
<td>-0.81</td>
<td>-0.86 to -0.76</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>0.27</td>
<td>0.25 to 0.30</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.12</td>
<td>0.050 to 0.18</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>-0.040</td>
<td>-0.070 to -0.011</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>-0.088</td>
<td>-0.11 to -0.061</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>-0.033</td>
<td>-0.060 to -0.0072</td>
</tr>
<tr>
<td>Height, m</td>
<td>0.035</td>
<td>-0.0023 to 0.073</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>0.17</td>
<td>0.14 to 0.19</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.14</td>
<td>0.11 to 0.17</td>
</tr>
<tr>
<td>Fasting glucose, m/dL</td>
<td>0.062</td>
<td>0.035 to 0.088</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>0.090</td>
<td>0.064 to 0.12</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.25</td>
<td>0.14 to 0.35</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.026</td>
<td>-0.031 to 0.083</td>
</tr>
<tr>
<td>Hypertension treatment</td>
<td>0.17</td>
<td>0.11 to 0.23</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.22</td>
<td>0.17 to 0.28</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease</td>
<td>0.048</td>
<td>-0.052 to 0.15</td>
</tr>
</tbody>
</table>

Model $R^2$ for baseline pulse amplitude, 29.3%; PAT ratio, 13.9%. Regression coefficients are for standardized (mean, 0; SD, 1) continuous variables. Age was adjusted for sex; sex was adjusted for age. Triglyceride and C-reactive protein levels were logarithmically transformed for analyses. Abbreviations as in Tables 1 and 2.
exposure to cardiovascular risk factors that may be reflected by structural changes, such as the enlargement of the brachial artery, and current endothelial reactivity, which may change quickly in response to the present environment. Changes in the latter are useful for the monitoring of short-term modifications related to interventions. Structural changes take longer to develop and, thus, may mirror long-standing cardiovascular risk factors. An important question is whether the weaker correlation of classical risk factors with hyperemic response variables might result from the fact that these variables provide additional risk information that is not captured by traditional risk factors.26

Increasing cardiovascular risk factor burden assessed by risk scores was associated with higher baseline measures in men and women. Correlations of baseline variables with Framingham risk score have been reported13,26 and were comparable with respect to the European Society of Cardiology score. Overall, women showed lower baseline values and stronger hyperemic response. The association of hyperemic response was strongest for FMD in women, whereas other measures did not show striking relations between the latter factors. Both weaker correlation of classical risk factors with hyperemic response variables did not change appreciably after accounting for age and sex. Importantly, correlations with hyperemic response variables did not change appreciably after accounting for age and sex. Thus, we can hypothesize that the correlation of vascular function measures with BMI indicates pathological changes that are not only accounted for by larger finger size.

### Fasting Blood Glucose and Microvascular Function

Interestingly, fasting blood glucose was markedly related to PAT. Although in human studies, impaired fasting glucose usually is not associated with overt microvascular dysfunction, animal models indicate changes in the microvasculature at early stages of impaired glucose metabolism.27 Hyperglycemia plays an important role in direct metabolic injury of endothelial cells; reduces vasoreactivity of small vessels; and induces vascular complications and end-organ damage, such as retinopathy, microalbuminuria, and neuropathy.28–30 Reduced hyperemic pulse amplitude may be an early indicator of microvascular impairment and end-organ changes. In our sample, the weaker association with FMD may be explained by a differing response to hyperemic stimulus in the 2 arterial beds. Although basal vascular tone is regulated by nitric oxide, and depletion of this mediator is an important cause of microvascular dysfunction,10,14 the role of nitric oxide is complex and differs depending on the arterial bed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reflection Index</th>
<th>Reflection Index Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.045</td>
<td>0.019 to 0.072</td>
</tr>
<tr>
<td>Female sex</td>
<td>−0.74</td>
<td>−0.80 to −0.69</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>−0.0055</td>
<td>−0.032 to 0.021</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.21</td>
<td>0.14 to 0.28</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>−0.037</td>
<td>−0.066 to −0.0079</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>0.014</td>
<td>−0.012 to 0.041</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>−0.34</td>
<td>−0.37 to −0.32</td>
</tr>
<tr>
<td>Height, m</td>
<td>−0.064</td>
<td>−0.10 to −0.026</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>0.041</td>
<td>0.013 to 0.068</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.019</td>
<td>−0.0082 to 0.045</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>−0.072</td>
<td>−0.099 to −0.045</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>−0.044</td>
<td>−0.071 to −0.018</td>
</tr>
<tr>
<td>Diabetes</td>
<td>−0.21</td>
<td>−0.31 to −0.11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>−0.079</td>
<td>−0.14 to −0.022</td>
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<td>Hypertension treatment</td>
<td>−0.040</td>
<td>−0.10 to 0.022</td>
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<tr>
<td>Dyslipidemia</td>
<td>0.090</td>
<td>0.032 to 0.15</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease</td>
<td>−0.028</td>
<td>−0.013 to 0.072</td>
</tr>
</tbody>
</table>

**Model R² for baseline reflection index, 15.8%; reflection index difference, 3.2%. Regression coefficients are for standardized (mean, 0; SD, 1) continuous variables. Age was adjusted for sex; sex was adjusted for age.**
Figure 2. Vascular function measures by sex-specific quartiles of European Society of Cardiology risk score in men and women. A, FMD; B, Volume plethysmographic PAT. C, Reflection index. P values were derived by Jonckheere-Terpstra trend test.
Limitations
An obvious limitation of the study in a population-based sample is the lack of a gold standard of invasive vascular function measurement, although for FMD, a correlation with invasive endothelial function has been demonstrated. Reproducibility of reflection index measurements was low and may account for the lack of correlations observed with cardiovascular risk factors. Because of ethical concerns in a predominantly healthy cohort, we did not administer the medication glyceryl trinitrate to assess nitroglycerin-mediated dilation, which provides additional information on the maximum, nonendothelium-dependent vasoreactivity and pulse waveform changes. We only measured vascular function once in every individual, which does not account for intraindividual variability and circadian rhythms. In addition, recent studies suggest that the maximal increase in diameter occurs >60 seconds after cuff release. The association of hyperemic vascular function measures registered at 60 seconds thus may not reflect the strongest relation with risk factors.

Among the strengths of our study in a large, well-characterized cohort, is the availability of noninvasive vascular function data simultaneously derived by 3 contemporary methods of testing. Differences in relations to classical cardiovascular risk factors may convey distinct pathophysiologies in conductance vessels and microvascular function. The large data set spanning a wide age range relevant for cardiovascular prevention may help to establish age-specific normal values and permit the assessment of vascular function in individuals without relevant cardiovascular risk factors. In addition, we clearly showed that the strongest predictors of vascular function across methods are age and sex and, to a lesser extent, BMI and hypertension, which may have implications for cardiovascular disease prevention.

Conclusions
We are only beginning to understand the implications of noninvasive vascular function testing. Experimental data are necessary to validate vascular function measures because at present, it is still unclear what exact part of vascular function the different methods capture and what physiological response can be expected. Robust epidemiological outcome data are needed to assess the value of structural and dynamic arterial changes for risk screening beyond classical risk factors. Finally, ease of use and cost will determine which vascular function measures may be applied in clinical practice.

Acknowledgments
We thank Thilo Weckmüller for his support in figure preparation.

Sources of Funding
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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Over the past years, several noninvasive measurement methods of vascular structure and reactivity have been implemented to assess cardiovascular function. We simultaneously measured flow-mediated dilation of the brachial artery, which reflects large artery reactivity, and finger pulse amplitude through volume and infrared plethysmography to assess microvascular function at baseline and after a 5-minute upper-arm occlusion in a population-based cohort. Vascular function measures showed low correlations among one another. The cardiovascular risk factors associated strongly with the most vascular function measures were age and sex followed by body mass index and indicators of blood pressure. Whereas baseline parameters were correlated with a broad spectrum of cardiovascular risk factors, the correlation with hyperemic response variables was weaker, and the overall variability explained by the risk factors was lower (maximum 15.4% for flow-mediated dilation). To understand the association of the 3 methods with cardiovascular disease outcomes, prospective data are needed to evaluate the value of structural and dynamic arterial changes for risk screening beyond classical risk factors.
Noninvasive Vascular Function Measurement in the Community: Cross-Sectional Relations and Comparison of Methods
Renate B. Schnabel, Andreas Schulz, Philipp S. Wild, Christoph R. Sinning, Sandra Wilde, Medea Eleftheriadis, Stephanie Herkenhoff, Tanja Zeller, Edith Lubos, Karl J. Lackner, Ascan Warnholtz, Tommaso Gori, Stefan Blankenberg and Thomas Münzel

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Supplemental Methods

Participant enrolment

Participants are invited for a clinic visit at the Center for Cardiovascular Prevention at the Johannes Gutenberg University Mainz. Fasting blood and anthropometric data are collected. Participants undergo standardized computer-assisted interviews on cardiovascular risk factors, lifestyle, and socioeconomic status. Medication intake is recorded by self-report. Participants are asked to bring their current medications. Classical cardiovascular risk factors were defined as follows: Smoking was dichotomized into non-smokers (never smokers and former smokers) and smokers (occasional smoker, i.e. <1 cigarette/day, and smoker, i.e. ≥1 cigarette/day). Diabetes mellitus was defined as individuals with a physician diagnosis of diabetes or a fasting blood glucose concentration of ≥126mg/dl after an overnight fast of at least 8 hours or a blood glucose level of ≥200mg/dl after a fasting period <8 hours. Dyslipidemia was defined as a physician diagnosis of dyslipidemia or an LDL/HDL-ratio of >3.5. Hypertension was diagnosed if anti-hypertensive drugs were taken, or a mean systolic blood pressure of ≥140mmHg or a mean diastolic blood pressure of ≥90mmHg (in the 2nd and 3rd standardized measurement after 8 and 11 minutes of rest) was measured. Prevalent cardiovascular disease was self-reported presence of any of the following: coronary artery disease, myocardial infarction, heart failure, or stroke. We used routine laboratory methods for blood glucose and lipid measurements. High sensitivity C-reactive protein was measured on an ARCHITECT® c8000, Abbott Diagnostics, Germany as a routine laboratory parameter by a standardized method.
**Supplemental Table 1.** Intra class correlation coefficients for the different vascular function measures in repeat measurements by two observers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intra-observer ICC Range</th>
<th>Inter-observer ICC Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline brachial artery diameter</td>
<td>0.72-1.0</td>
<td>0.87-0.95</td>
</tr>
<tr>
<td>Hyperemic brachial artery diameter</td>
<td>0.87-0.93</td>
<td>0.90-0.93</td>
</tr>
<tr>
<td>Baseline reflection index</td>
<td>0.03-0.75</td>
<td>-0.45- -0.14</td>
</tr>
<tr>
<td>Hyperemic reflection index</td>
<td>0.53-0.83</td>
<td>0.19-0.29</td>
</tr>
<tr>
<td>Baseline pulse amplitude</td>
<td>0.89-0.90</td>
<td>0.54-0.63</td>
</tr>
<tr>
<td>PAT ratio</td>
<td>0.95-0.96</td>
<td>0.62-0.67</td>
</tr>
</tbody>
</table>

ICC indicates intra class correlation coefficient.
**Supplemental Table 2.** Baseline characteristics by sign of hyperemic reflection index difference

<table>
<thead>
<tr>
<th>Variable</th>
<th>Increase N=1960</th>
<th>Decrease N=2789</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>56.6±10.7</td>
<td>54.1±10.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>135.2±17.3</td>
<td>132.1±17.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.1±8.9</td>
<td>78.9±9.0</td>
<td>0.48</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>64.2±10.9</td>
<td>64.4±9.9</td>
<td>0.51</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.71±0.09</td>
<td>1.70±0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8 (24.2/30.0)</td>
<td>26.3 (23.6/29.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>4.24±1.21</td>
<td>4.18±1.25</td>
<td>0.098</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>110.0 (81.0/153.4)</td>
<td>104.4 (77.4/146.0)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>92.2 (86.0/99.2)</td>
<td>90.2 (85.0/97.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.7 (0.5/3.3)</td>
<td>1.6 (0.5/3.2)</td>
<td>0.52</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>169 (8.6%)</td>
<td>180 (6.5%)</td>
<td>0.0057</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>370 (18.9%)</td>
<td>551 (19.8%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>1080 (55.1%)</td>
<td>1316 (47.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antihypertensive treatment, n (%)</td>
<td>643 (32.8%)</td>
<td>684 (24.5%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>571 (29.1%)</td>
<td>808 (29.0%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Prevalent CVD, n (%)</td>
<td>173 (8.8%)</td>
<td>192 (6.9%)</td>
<td>0.016</td>
</tr>
<tr>
<td>Family history for CVD, n (%)</td>
<td>347 (17.7%)</td>
<td>498 (17.9%)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Vascular function measures**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Increase N=1960</th>
<th>Decrease N=2789</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pulse amplitude</td>
<td>6.10±0.88</td>
<td>6.01±0.92</td>
<td>0.0016</td>
</tr>
<tr>
<td>PAT ratio</td>
<td>0.53±0.44</td>
<td>0.57±0.45</td>
<td>0.0016</td>
</tr>
<tr>
<td>Baseline diameter brachial artery</td>
<td>4.40±0.80</td>
<td>4.27±0.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.82±4.78</td>
<td>8.25±4.95</td>
<td>0.0032</td>
</tr>
<tr>
<td>European Society of Cardiology Score</td>
<td>3.00 (1.00/6.33)</td>
<td>2.00 (1.00/5.00)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Continuous variables are described by median, 25th and 75th percentile, if they had a skewed distribution (|skewness| > 1). Normally distributed variables are presented as mean values and standard deviation. Discrete variables are described through relative and absolute frequencies. Discrete Variables were tested with Chi-square-Test for contingency tables; continuous variables were analysed with Student-T-Test if they were normally distributed and with Mann-Whitney-U-Test on skewed distribution.

BMI stands for body-mass-index, FMD for flow-mediated dilation, HDL for high density lipoprotein, and PAT for peripheral arterial tonometry.
**Supplemental Table 3.** Limits of vascular function measures in the reference group by age (<50 and ≥50 years).

<table>
<thead>
<tr>
<th></th>
<th>Percentile</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>5&lt;sup&gt;th&lt;/sup&gt;</th>
<th>25&lt;sup&gt;th&lt;/sup&gt;</th>
<th>50&lt;sup&gt;th&lt;/sup&gt;</th>
<th>75&lt;sup&gt;th&lt;/sup&gt;</th>
<th>95&lt;sup&gt;th&lt;/sup&gt;</th>
<th>99&lt;sup&gt;th&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men &lt;50 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline brachial</td>
<td>3.17</td>
<td>3.71</td>
<td>4.22</td>
<td>4.60</td>
<td>4.95</td>
<td>5.42</td>
<td>6.00</td>
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</tr>
<tr>
<td>artery diameter, mm</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow-mediated dilation</td>
<td>0.49</td>
<td>1.30</td>
<td>3.63</td>
<td>5.81</td>
<td>8.57</td>
<td>13.87</td>
<td>17.59</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Baseline pulse</td>
<td>3.97</td>
<td>4.77</td>
<td>5.67</td>
<td>6.28</td>
<td>6.83</td>
<td>7.26</td>
<td>7.46</td>
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<td>amplitude</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PAT ratio</td>
<td>-0.64</td>
<td>-0.03</td>
<td>0.24</td>
<td>0.49</td>
<td>0.76</td>
<td>1.05</td>
<td>1.32</td>
<td></td>
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<tr>
<td>Baseline reflection</td>
<td>37.09</td>
<td>44.00</td>
<td>63.00</td>
<td>74.00</td>
<td>81.00</td>
<td>91.00</td>
<td>95.45</td>
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<tr>
<td>Reflexion index</td>
<td>-34.00</td>
<td>-20.75</td>
<td>-8.00</td>
<td>-2.00</td>
<td>3.08</td>
<td>19.75</td>
<td>28.48</td>
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<td></td>
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<tr>
<td>Men ≥50 years</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline brachial</td>
<td>2.91</td>
<td>3.72</td>
<td>4.59</td>
<td>4.96</td>
<td>5.30</td>
<td>5.82</td>
<td>6.11</td>
<td></td>
</tr>
<tr>
<td>artery diameter, mm</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Flow-mediated dilation</td>
<td>0.52</td>
<td>1.61</td>
<td>4.18</td>
<td>6.77</td>
<td>9.14</td>
<td>12.72</td>
<td>20.96</td>
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<tr>
<td>%</td>
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<tr>
<td>Baseline pulse</td>
<td>4.03</td>
<td>4.89</td>
<td>5.93</td>
<td>6.59</td>
<td>6.97</td>
<td>7.43</td>
<td>7.64</td>
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<td></td>
</tr>
<tr>
<td>PAT ratio</td>
<td>-0.19</td>
<td>-0.06</td>
<td>0.16</td>
<td>0.38</td>
<td>0.70</td>
<td>1.12</td>
<td>1.45</td>
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<tr>
<td>Baseline reflection</td>
<td>28.74</td>
<td>45.90</td>
<td>69.00</td>
<td>76.00</td>
<td>83.00</td>
<td>90.00</td>
<td>96.26</td>
<td></td>
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<tr>
<td>index</td>
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<tr>
<td>Reflexion index</td>
<td>-38.11</td>
<td>-12.30</td>
<td>-5.00</td>
<td>0</td>
<td>5.00</td>
<td>20.15</td>
<td>30.76</td>
<td></td>
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<td>difference</td>
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<td>Women &lt;50 years</td>
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<td>3.85</td>
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<td>4.95</td>
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<td>Flow-mediated dilation</td>
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<td>2.58</td>
<td>7.14</td>
<td>11.45</td>
<td>14.81</td>
<td>23.16</td>
<td>24.60</td>
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<td>Baseline pulse</td>
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<td>3.91</td>
<td>4.85</td>
<td>5.43</td>
<td>6.03</td>
<td>6.66</td>
<td>7.05</td>
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<td>-0.01</td>
<td>0.54</td>
<td>0.77</td>
<td>1.00</td>
<td>1.44</td>
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<td>62.00</td>
<td>72.00</td>
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<td>93.38</td>
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<td>Reflexion index</td>
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<td>Men ≥50 years</td>
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<td><strong>Baseline brachial artery diameter, mm</strong></td>
<td>2.58</td>
<td>2.92</td>
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<td><strong>Flow-mediated dilation, %</strong></td>
<td>0.35</td>
<td>1.66</td>
<td>5.53</td>
<td>8.20</td>
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<td>17.82</td>
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<td>-0.08</td>
<td>0.31</td>
<td>0.66</td>
<td>0.96</td>
<td>1.46</td>
<td>1.91</td>
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<tr>
<td><strong>Baseline reflection index</strong></td>
<td>26.02</td>
<td>43.70</td>
<td>54.00</td>
<td>63.00</td>
<td>72.00</td>
<td>83.00</td>
<td>87.00</td>
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<td>-44.37</td>
<td>-28.00</td>
<td>-11.00</td>
<td>-3.00</td>
<td>5.00</td>
<td>20.00</td>
<td>28.18</td>
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### Supplemental Table 4. Spearman correlation coefficients between BMI and vascular function measures

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<tr>
<th></th>
<th>Baseline diameter</th>
<th>FMD</th>
<th>Baseline pulse amplitude</th>
<th>PAT ratio</th>
<th>Baseline reflection index</th>
<th>Reflection index difference</th>
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<tr>
<td>BMI</td>
<td>0.34</td>
<td>-0.18</td>
<td>0.36</td>
<td>-0.28</td>
<td>0.089</td>
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<td>BMI adjusted for age and sex</td>
<td>0.28</td>
<td>-0.10</td>
<td>0.29</td>
<td>-0.23</td>
<td>0.013</td>
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<tr>
<td>BMI adjusted for age, sex and finger girth</td>
<td>0.21</td>
<td>-0.095</td>
<td>0.25</td>
<td>-0.20</td>
<td>-0.017</td>
<td>0.043</td>
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</tbody>
</table>

FMD indicates flow-mediated dilation, PAT indicates peripheral arterial tonometry, RI stands for reflection index difference
Supplemental Figures and Figure Legends

Supplemental Figure 1. Vascular function measures by 10-year increments in age in the reference sample. P values are for the Jonckheere-Terpstra trend test.

Flow-mediated dilation
Volume plethysmographic peripheral arterial tonometry

![Box plots showing Baseline Pulse Amplitude and PAT Ratio by age decades and gender.](image)

- **Baseline Pulse Amplitude**:
  - Men: P for trend: <0.0001
  - Women: P for trend: <0.0001

- **PAT Ratio**:
  - Men: P for trend: 0.93
  - Women: P for trend: 0.52

N: 126, 142, 78, 45, 233, 150, 109, 63
Reflection index

![Graph showing baseline reflection index and reflection index difference by age decades for men and women.](image)

**Baseline Reflection Index**
- Men: N = 126, N = 152, N = 84, N = 50
- Women: N = 257, N = 161, N = 118, N = 70

**P for trend**
- Men: 0.022
- Women: 0.052

**Reflection Index Difference**
- Men: N = 124, N = 151, N = 84, N = 49
- Women: N = 255, N = 158, N = 115, N = 67

**P for trend**
- Men: 0.25
- Women: 0.010

---

*Note: The graphs illustrate the distribution of baseline reflection index and reflection index difference across different age decades for men and women. The statistical significance (P for trend) indicates the trend observed in the data across age decades for each gender.*
Supplemental Figure 2. Summary scheme of statistically significant bivariate correlations between vascular function measures and cardiovascular risk factors in relation to the vascular bed they are derived from. The following abbreviations were used: BMI, body-mass-index; diastolic BP, diastolic blood pressure; FMD, flow-mediated dilation; PAT, volume plethysmographic peripheral arterial tonometry; Ri diff, reflection index difference; systolic BP, systolic blood pressure.
Supplemental Figure 3. Boxplots of vascular function measures in relation to normal fasting glucose, impaired glucose tolerance, and manifest diabetes mellitus. P values are for the Jonckheere-Terpstra trend test.

Flow-mediated dilation
Volume plethysmographic peripheral arterial tonometry

![Graph showing baseline pulse amplitude and PAT ratio across different glucose statuses with statistical significance.](image)