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Is the Association Between Flow-Mediated Dilation and Cardiovascular Risk Limited to Low-Risk Populations?

Daniel R. Witte, MD, PHD,* Jan Westerink, MD,† Eelco J. de Koning, MD, PHD,†‡
Yolanda van der Graaf, MD, PHD,* Diederick E. Grobbee, MD, PHD,* Michiel L. Bots, MD, PHD*
Utrecht and Leiden, the Netherlands

OBJECTIVES The aim of this research was to study whether the relation between endothelial function measured by flow-mediated dilation (FMD) of the brachial artery and cardiovascular risk factors is affected by the baseline cardiovascular risk.

BACKGROUND Flow-mediated dilation of the brachial artery is widely used as a measure of endothelial function. Relations between FMD and most cardiovascular risk factors have been described.

METHODS We performed a meta-regression analysis of 211 selected articles (399 populations) reporting on FMD and cardiovascular risk factors. Mean values of FMD; age; proportion of men; proportion of smokers; blood pressure; lipids; glucose; and the presence of diabetes mellitus, of hyperlipidemia, and of hypertension were retrieved from the articles. The 10-year risk of coronary heart disease (CHD) for each population was estimated based on the Framingham risk score. The relation between FMD and cardiovascular risk factors was assessed within each risk category by linear regression analysis, adjusting for age and gender, and weighted for the study size.

RESULTS A relation between FMD and cardiovascular risk factors was most clear in the category with lowest baseline risk (below 2.8% per decade). In populations with low baseline risk, for each % increase in Framingham risk, FMD decreased by 1.42% (95% confidence interval: 0.65 to 2.19). In medium- and high-risk populations, FMD was not related to risk (−0.02% [−0.27 to 0.22] and 0.06% [−0.02 to 0.13], respectively). These findings were independent of differences in brachial lumen diameter and technical aspects of the FMD measurement.

CONCLUSIONS Only in populations at low risk, endothelial function measured by FMD is related to the principal cardiovascular risk factors, and to the estimated 10-year risk of CHD. (J Am Coll Cardiol 2005;45:1987–93) © 2005 by the American College of Cardiology Foundation

Endothelial dysfunction is recognized as a major factor in the development of atherosclerosis (1,2). Assessment of endothelial function has long been limited by the invasive nature of measurements. In 1992 Celermajer et al. (3) proposed a noninvasive method to assess endothelial function (flow-mediated dilation [FMD]). This measurement assesses the brachial artery diameter by ultrasound before and after a period of induced ischemia of the forearm. Ischemia is induced by inflation of a blood pressure cuff placed on the distal part of the arm, or on the proximal arm; FMD is generally expressed as the percentage increase in brachial artery diameter after release of occlusion. This dilation is mediated by endothelial nitric oxide (NO) release (4) in response to increased shear stress (5). Thus, FMD is thought to reflect the endothelial NO-mediated regulation of vascular tone and diameter.

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The introduction of FMD opened the possibility of measuring endothelial function with minimal burden. Application of it to populations at large and the pivotal role of endothelium in early atherogenesis (1) led to great expectations. Flow-mediated dilation was expected to be of use in risk stratification and in the assessment of the effectiveness of therapy (6). Although numerous studies showed a relation between FMD and cardiovascular risk factors (7,8) and diseased or compromised patients (9–12), evidence for the prognostic value of FMD was lacking until recently. Modena et al. (13) showed that persistently decreased brachial endothelial function relates to a higher incidence of nonfatal cardiovascular events in hypertensive postmenopausal women (based on 32 events). Gökce et al. (14) reported that brachial artery endothelial function predicts postoperative events in patients undergoing vascular surgery (based on 45 events). Surprisingly, a longer event-free survival was seen only in the upper tertile of FMD (>8.1%) (14). Previously, Rubenfire et al. (15) reported that the difference in FMD between individuals at average risk and those at high risk was almost three times larger than the difference in FMD between those at high risk and patients with clinically manifest coronary artery disease. These observations could imply that the relation between brachial artery endothelial function and cardiovascular risk is not the same for all individuals but may differ according to baseline risk. To explore this concept further, we studied the relation between brachial artery endothelial function and cardiovascular risk factors across populations, stratified by cardiovascular risk.

From the *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; †Department of Internal Medicine, Section of Vascular Medicine, Diabetes and Endocrinology, University Medical Center Utrecht, Utrecht, the Netherlands; and the ‡Department of Internal Medicine, Section of Nephrology, Leids University Medical Center, Leiden, the Netherlands.

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Abbreviations and Acronyms
CHD = coronary heart disease
FMD = flow-mediated dilation
NO = nitric oxide

METHODS

Search strategy. Data were obtained from published papers reporting FMD values. We performed a literature search on PubMed. Table 1 shows the four separate queries that were used. All queries were limited to "English language," "human study," and publication date between January 1, 1992, and December 31, 2002. The search was performed on January 10, 2003, and yielded 832 hits. Abstracts of these papers were reviewed for "FMD of the brachial artery by ultrasound imaging," and 348 papers were excluded. The full text copies of the remaining 484 papers were retrieved and reviewed. Papers were selected if they at least reported: mean FMD, expressed as a percentage, with a corresponding standard deviation or standard error; population size; and information on cardiovascular risk factors.

Selection of papers. These criteria led to the exclusion of 273 papers. Twenty reports were excluded because of no report on brachial FMD, 10 because of reviews not providing original data, 3 because of a double report on the same population (the most complete report was included). A further 49 reports were excluded due to unclear presentation of results (multiple ill-defined subgroups, data presented only in figures, no standard error or standard deviation for FMD, or FMD not expressed as a percentage), and 191 papers were excluded because they did not provide most of the required baseline characteristics. Thus, the final set consisted of 211 papers (Appendix). If a paper reported on more than one population or well-defined population subset, each of these was entered and regarded as a separate population. The 211 selected papers provided data on 399 populations, which served as the basis for the main analyses.

Table 1. MEDLINE Queries Used to Retrieve Papers on FMD

Query	Search Terms	Number of Hits
#1	"brachial artery" AND ("endothelium" OR "endothelial") AND ("function" OR "dysfunction")	598
#2	("FMD" OR "vascular reactivity") AND "brachial artery"	257
#3	"flow mediated" AND ("vasodilatation" OR "vasodilation" OR "dilatation" OR "dilation")	521
#4	"endothelium dependent" AND ("vasodilatation" OR "vasodilation" OR "dilatation" OR "dilation") AND "brachial artery"	407
	Combined (#1 OR #2 OR #3 OR #4)	832

All queries were limited to "English language," "human study," and publication date between January 1, 1992 and December 31, 2002.
FMD = flow-mediated dilation.

Data entry. For each population, the mean FMD with its standard error; the mean nitroglycerin response with its standard error; baseline diameter of the brachial artery; the number of participants; proportion of males; mean age; body mass index; lipids; blood pressure; glucose; and proportion of participants with diabetes mellitus, hypertension, or hypercholesterolemia were retrieved from the publication and entered into a database by two of the authors (J.W. and D.R.W.). In a subset of the papers (208 populations), information had been extracted on technical aspects relating to the FMD measurement, including information on type of equipment (wall track/B-mode), location of the measurement (antecubital fossa/upper arm), occlusion site (upper/lower arm), occlusion duration (min), and occlusion pressure. This dataset has been used for a separate paper with a focus on technical aspects of the FMD measurement as determinants of FMD response (16).

Missing values. In 171 papers (293 populations), between one and three of the variables high-density lipoprotein, systolic or diastolic blood pressure, or glucose were not available from the text or tables. Systolic blood pressure was missing in 108 populations, and diastolic blood pressure in 116 populations. These studies all provided the proportion of subjects with hypertension. High-density lipoprotein cholesterol was missing in 59 populations, and glucose was missing in 247 populations. All papers with missing high-density lipoprotein provided the proportion of patients with hyperlipidemia, and all papers with missing glucose values provided the proportion of patients with diabetes mellitus. To avoid the potential problem of underestimating the Framingham risk score in populations with missing data due to assignment of the lowest risk category in case of missing information, we estimated the missing values by imputation with the "regression imputation method" (17). Based on the populations with complete data, four separate linear regression models were fitted, for systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol, and glucose as dependent variables. Independent variables were proportion of men, proportion of smokers, age, and total cholesterol in all models. The model for high-density lipoprotein cholesterol, systolic and diastolic blood pressure, and glucose also included the proportion of participants with hyperlipidemia, hypertension, and diabetes mellitus, respectively. Additionally, the respective quadratic terms of the independent variables were also included in the models. The linear regression models yielded a R^2 of 0.41, 0.60, 0.42, and 0.79 for high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, and glucose, respectively. Models were used to calculate estimates for the missing high-density lipoprotein cholesterol, glucose, and systolic and diastolic blood pressure values to replace the missing values.

Estimation of cardiovascular risk. For each study population, the 10-year risk of coronary heart disease (CHD) was estimated according to the Framingham risk score (18).

Table 2. Patient Characteristics of the Studied Populations, by Tertiles of Framingham Risk

		Tertiles of Framingham Risk Score					
	n	Low-Risk		Medium-Risk		High-Risk	
Males (%)	399	46.8	30.0	55.5	33.7	78.4	15.8
Mean age (yrs)	399	30.4	6.5	50.6	8.3	57.5	4.0
Body mass index (kg/m^2)	250	23.6	2.5	24.5	2.0	25.8	2.3
Total cholesterol (mmol/l)	399	4.9	0.7	5.4	0.7	5.0	0.7
LDL cholesterol (mmol/l)	291	3.0	0.8	3.4	0.6	3.1	0.7
HDL cholesterol (mmol/l)*	399	1.4	0.1	1.4	0.2	1.1	0.2
Triglycerides (mmol/l)	320	1.0	0.3	1.5	0.6	1.8	0.5
Glucose (mmol/l)*	399	5.7	1.7	5.1	0.6	5.7	1.1
HbA1c (%)	59	7.7	1.9	5.1	0.7	6.4	1.5
Homocysteine (μmol/l)	43	9.9	3.0	12.2	7.3	11.0	1.5
Systolic blood pressure (mm Hg)*	399	116.8	4.6	123.8	7.0	129.7	5.8
Diastolic blood pressure (mm Hg)*	399	71.0	4.9	77.0	4.6	77.4	3.7
Heart rate (beats/min)	120	69.1	5.3	66.9	5.6	61.8	3.4
Current smokers (%)	399	11.0	23.1	19.3	23.9	29.7	27.9
Diabetes mellitus (%)	379	13.0	33.7	1.8	12.4	13.1	24.1
Hyperlipidemia (%)	271	3.8	19.1	7.5	20.5	31.4	32.3
Hypertension (%)	336	0.1	0.7	5.9	17.1	34.6	20.1
Coronary artery disease (%)	284	0.0	0.0	5.5	21.1	68.6	45.9
FMD (% increase of baseline diameter)	399	6.8	3.5	5.4	2.7	4.5	2.1
NTG (% increase of baseline diameter)	305	17.1	3.7	16.0	3.2	14.9	3.5
Baseline diameter (mm)	333	3.5	0.5	4.0	0.6	4.3	0.3
Baseline brachial artery flow (ml/min)	143	91.8	60.9	184.2	81.1	128.4	78.0
Reactive hyperemia (% of baseline flow)	190	522.3	224.1	398.0	133.4	336.4	130.3
Framingham risk score (% risk/10 yrs)	399	1.1	0.8	6.6	1.9	13.5	4.7

Values are means (SD). *Values for high-density lipoprotein (HDL) cholesterol, glucose, systolic and diastolic blood pressure include imputed values.
FMD = flow-mediated dilation; LDL = low-density lipoprotein; NTG = nitroglycerin.

Instead of the dichotomous categories for gender and smoking, the proportions of men and smokers were used.

Analysis. We used meta-regression techniques (19,20). Yet, our approach differs in two ways. Firstly, our outcome (dependent) variable was not a measure of association (e.g., an odds ratio), but the mean reported FMD value. Secondly, we did not aim at estimating a common "true" mean FMD value over all studies. We instead assumed the existence of heterogeneity and explored some of the possible sources of variability between studies. For this reason we have chosen a "fixed effects" rather than a "random effects" model.

All analyses were weighted according to the inverse variance of the FMD measurement: $1/[\mathrm{SE}(\mathrm{FMD})]^2$. Because in the SE of the FMD the number of subjects is included, this approach also assigns greater weights to larger studies and smaller weights to smaller studies. We first explored the relation between FMD and Framingham risk in a univariate manner with Lowess regression (locally weighted polynomial regression) and compared a linear model with a model including a quadratic term for Framingham risk. Populations were then categorized according to tertiles of Framingham risk score (cutoff points were score of 2.8 and 9.5). Within each risk category (low, medium, and high risk), the relation between FMD and individual risk factors was explored by linear regression, adjusting for age and gender, and weighted by study size. The presence of effect modification of these relations by cardiovascular risk was tested by linear regression in the presence of a multiplicative interaction term. Similar analyses were performed for the nitroglycerine response.

RESULTS

The 399 populations included in our analyses reported on 11,984 patients. Population size varied from 5 to 654 (median 20). General characteristics by category of Framingham risk are presented in Table 2. The Lowess regression line (Fig. 1) shows how the local regression line changes with changing baseline risk. A linear regression model of the relation between Framingham risk (independent) and FMD (dependent), including a quadratic and a cubic term for Framingham risk, yielded the following equation:

$$\mathrm{FMD}(\%) = 7.226 - 0.4170 \cdot \mathrm{FR} + 0.0195 \cdot \mathrm{FR}^2 - 0.0003 \cdot \mathrm{FR}^3$$

All terms were statistically significant ($p < 0.001$, $p = 0.012$, and $p = 0.046$ for the linear, quadratic, and cubic terms, respectively). This regression line shows an initial steep negative slope, which gradually changes towards a flat line as risk increases. In other words, with increasing risk, the magnitude of the relation between FMD and risk gradually attenuates. The coefficients did not materially change in magnitude nor direction and significance when adjustments were made for baseline lumen diameter, for location of the occlusion cuff (upper arm/lower arm), or for occlusion time (<4 min/5 min). Interaction terms for the

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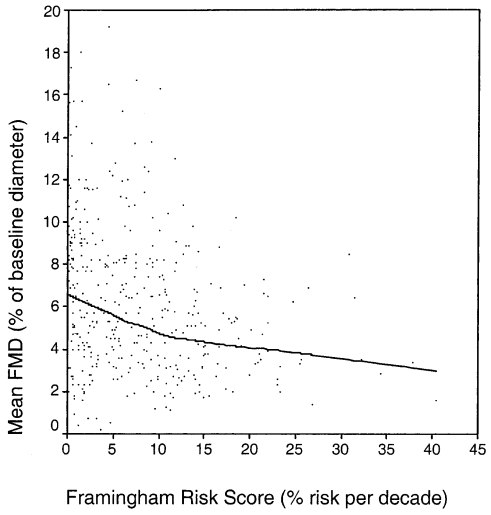

Figure 1. Plot of the mean flow-mediated dilation (FMD) of the brachial artery by mean Framingham risk estimate for each of the study populations. **Line** is the Lowess regression line. **Points** are weighted according to the inverse variance of FMD for each study [1/SE (FMD)^2]. Tertiles of Framingham risk cutoff points were 2.8% and 9.5%.

technical aspects were not statistically significant. This implies that the relation of FMD and Framingham risk score in subjects with an upper arm cuff position was similar to the relation found in subjects with a lower arm cuff position. However, when year of publication was taken into account as a means to further adjust for between-study differences, the p value of the cubic term attenuated to 0.11.

Given the importance of cuff location and the fact that 81% of the studies used lower arm occlusion, we analyzed the data in that group separately based on data from 169 studies despite the absence of a statistically significant interaction term. The regression coefficients of the model were:

$$\text{FMD}(\%) = 6.453 - 0.369 \cdot \text{FR} + 0.021 \cdot \text{FR}^2 - 0.00035 \cdot \text{FR}^3$$

The statistical significance of the parameters were $p = 0.007$, $p = 0.047$, and $p = 0.082$ for the linear, quadratic, and cubic terms, respectively. This regression line looks similar to the earlier one in that it shows an initial negative slope, which gradually changes towards a flat line as risk increases. The analysis among populations with upper arm occlusion ($n = 39$) showed a formula of $\text{FMD}(\%) = 14.246 - 1.518 \cdot \text{FR} + 0.001 \cdot \text{FR}^2 - 0.000024 \cdot \text{FR}^3$ with statistical significance values of $p = 0.03$, $p = 021$, and $p = 0.46$. This subgroup analysis, with very limited power and, thus, limited precision for detecting trends different than linear trends, indicated a linear decline in FMD with increasing Framingham risk score.

Figure 2 shows that a significant inverse relation between the Framingham risk score and FMD is only present when risk score is below three points. No changes were seen when the model was additionally adjusted for baseline lumen diameter, for location of the occlusion cuff, or occlusion time. When year of publication was taken into account, the significance and magnitude of the relations was not altered.

The relation between cardiovascular risk factors and the mean FMD value within each cardiovascular risk category is displayed in Table 3. In populations at low risk, an inverse relation with FMD is clearly present for most cardiovascular risk factors. In populations at intermediate or high risk, most relations are of a lower magnitude or absent. The last column shows the p values for the multiplicative interaction terms. Significant interaction was present for age, total cholesterol, low-density lipoprotein cholesterol, glucose, and proportion of smokers, indicating that, for these variables, the relation with FMD was significantly different across the three strata of risk.

The results for nitroglycerine response were similar to the findings for FMD:

$$\text{NTG}(\%) = 10.84 - 1.241 \cdot \text{FR} + 0.07 \cdot \text{FR}^2 - 0.001 \cdot \text{FR}^3$$

All terms were statistically significant ($p < 0.001$, $p = 0.001$, and $p = 0.008$ for the linear, quadratic, and cubic terms, respectively). The relations of Framingham risk score with nitroglycerine in tertiles of risk score showed a strong inverse relation among the low-risk group, and less strong relations for the middle- and upper-risk score tertiles. The regression coefficients reflecting change in nitroglycerine response with one point increase in Framingham risk score were −4.31% (95% confidence interval −5.72 to −2.90), 0.07% (−0.42 to 0.55), and −0.31% (−0.41 to −0.21), respectively.

DISCUSSION

Our analysis showed that endothelial function, as measured by FMD, is related to the principal cardiovascular risk factors, and to the estimated 10-year risk of CHD predom-

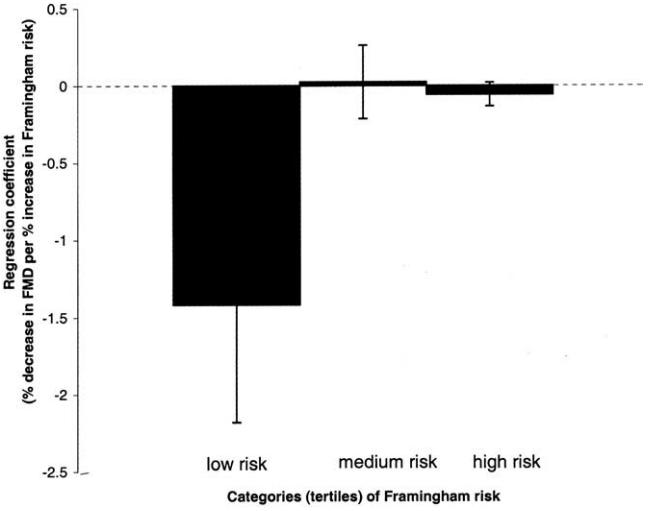

Figure 2. The association between Framingham risk and flow-mediated dilation (FMD) of the brachial artery within tertiles of Framingham risk, with 95% confidence intervals.

Table 3. Relation Between Endothelial Function and Cardiovascular Risk Factors, by Tertiles of Cardiovascular Risk

		Low-Risk		Medium-Risk		High-Risk		
	n	β	95% CI	β	95% CI	β	95% CI	p(int)
Males (per 10%)	399	−0.35	−0.55; −0.15	−0.08	−0.24; 0.07	−0.14	−0.38; 0.10	0.09
Age (per decade)	399	−0.60	−1.52; 0.32	0.49	−0.15; 1.12	0.51	−0.45; 1.46	0.00
BMI (per kg/m^2)	250	−0.24	−0.50; 0.02	0.04	−0.28; 0.36	−0.38	−0.53; −0.23	0.15
Total cholesterol (mmol/l)	399	−1.36	−2.18; −0.53	−0.83	−1.48; −0.19	0.53	−0.03; 1.09	0.01
LDL cholesterol (mmol/l)	291	−1.36	−2.31; −0.40	−0.75	−1.72; 0.22	0.47	−0.20; 1.13	0.01
HDL cholesterol (mmol/l)	399	−1.43	−5.89; 3.04	−0.13	−2.84; 2.57	3.19	1.03; 5.36	0.56
Triglycerides (mmol/l)	320	−1.81	−4.27; 0.65	−1.12	−1.93; −0.31	0.16	−0.75; 1.07	0.57
Glucose (mmol/l)	399	−0.64	−0.99; −0.29	−0.06	−0.90; 0.78	−0.05	−0.38; 0.29	0.03
HbA1c (%)	59	−1.13	−1.73; −0.53	−0.28	−1.93; 1.37	−0.69	−1.47; 0.09	0.23
Homocysteine (μmol/l)	43	−1.00	−2.18; 0.19	−0.03	−0.17; 0.11	−0.14	−0.72; 0.45	0.78
Systolic BP (per 10 mm Hg)	399	−0.65	−2.11; 0.81	−0.41	−1.06; 0.24	−1.23	−1.83; −0.63	0.09
Diastolic BP (per 10 mm Hg)	399	−1.94	−3.37; −0.50	−0.22	−1.24; 0.80	−0.78	−1.77; 0.20	0.08
Smokers (per 10%)	399	−0.26	−0.51; 0.00	−0.14	−0.34; 0.06	−0.08	−0.21; 0.05	0.01

Regression coefficients are weighted according to the inverse variance of flow-mediated dilation for each study [1/SE(FMD)^2] and adjusted for age and gender. Regression coefficients for gender and age are adjusted for the other. n indicates the number of populations included. p(int) indicates the p value of the multiplicative interaction term using risk factor and risk score as continuous variables.

BMI = body mass index; BP = blood pressure; CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

inantly in populations with a low baseline risk. A similar finding was observed for the nitroglycerine response.

Before we accept our findings, a number of issues need to be addressed. First, we performed meta-regression analysis to explore baseline risk as a source of heterogeneity in the relation between FMD and cardiovascular risk factors across different populations. The most important advantage of our approach is that it enables investigation of the relation between FMD and risk across a far wider range of risk than would be possible within one single study. Most studies reporting on FMD examine a very limited number (median 20) of highly selected patients. As a consequence, the variation in baseline cardiovascular risk within each population is small. In order to obtain the wide range of baseline risk required to address our research question, we have compiled a population of study populations. In most meta-analyses, the aim is to summarize a measure of effect into one single estimate of the "true" effect. In such a situation, heterogeneity between studies is a potential source of bias that needs to be minimized. In our case, however, virtually none of the existing studies reported an estimate of the relation between FMD and cardiovascular risk. We have, therefore, chosen to study this relation based on the variation in FMD and cardiovascular risk between studies. Consequently, our aim was not to estimate one single "true" mean FMD, but to explain the variation in FMD by the variation in cardiovascular risk. Second, because information on some risk factor variables could not be extracted from a large number of papers, the missing values were imputed. There is ample evidence indicating that imputation of missing values is preferable over analysis of fully informative cases only (16), the latter leading to biased results. We have imputed only the missing values of the variables needed to calculate the Framingham risk score, thereby avoiding the underestimation of risk in populations with missing values that would have occurred had we calculated the Framingham risk score without prior imputation. Third, when making inferences, results may be a consequence of confounding. We have dealt with this issue by adjusting our analyses for the mean age and the gender of each population in the analyses of separate risk factors. Moreover, adjustment for details of the FMD study protocol did not alter our principal findings. Finally, it should be noted that comparison of the results of the within-tertile analyses might be hampered by differences in risk factor distribution between the tertiles, and with that a different a priori power to detect a relationship with FMD in a stratum.

Our main finding (i.e., the absence of a relation between FMD and cardiovascular risk in populations at medium and high risk) could be explained in two distinct ways. First is that the observations are the result of a biological phenomenon. Damage to the endothelium and the resulting endothelial dysfunction is thought to be one of the initial steps in atherogenesis (1,21,22). It is conceivable that the ability of endothelium to produce NO in response to stimuli like shear stress is affected at an early stage in this process and that beyond this point additional increase in risk factors does not cause an additional decline of function. According to this view, other measures of endothelial function such as adhesion molecules (intracellular adhesion molecule [ICAM-1], vascular cell adhesion molecule [VCAM-1], e-selectin) or invasive measures of endothelial function should yield similar results. Although no similar meta-analyses exist for other measures of endothelial function, circumstantial evidence suggests that there is a continuous relation in populations at high risk. A meta-analysis of serum markers of endothelial function (ICAM and VCAM), based on generally healthy populations (23), showed a relation with cardiovascular risk, but did not examine this relation separately in categories of increasing risk. Invasive measures of endothelial function with a pharmacological stimulus have been found to be related to

prospective cardiovascular risk (24,25) in patients at medium or high risk.

A second possible explanation might be that the accuracy of ultrasonographic assessment of FMD as a measure of endothelial function is hampered in subjects with increased risk. More specifically, vessels, including the brachial artery, are known to become progressively stiffer with age and increasing cardiovascular risk factors (26). If arterial stiffness poses a physical limit to the ability of the brachial artery to dilate, even in the presence of functional endothelium, this would mean that, in high-risk patients (with stiffer arteries), FMD is no longer a reflection of endothelial NO-related mechanisms, but is distorted by stiffness and may become difficult to interpret. Our findings on nitroglycerine values support this notion. In addition, we have previously observed in a population of high-risk patients that the relation between FMD and age and smoking was present only in patients with relatively preserved distensibility of the brachial artery (27). Furthermore, the lack of a relation between the Framingham risk score and FMD in those at high risk of cardiovascular disease as found in our study may indicate that, indeed, in high-risk patients, the measured FMD might not reflect endothelial function completely.

In contrast with our findings may be the observations by Gökce et al. (14) who showed in a very high-risk group that endothelial dysfunction does relate to an increased risk of cardiovascular events. A longer event-free survival was only observed in the upper tertile of FMD (>8.1%), whereas middle and low tertiles of FMD showed no difference in event-free survival (14). Based on the earlier mentioned arguments, it may be that the described risk relations reflect another process than endothelial function per se. Please note that the relationship with patient-averages across studies may not be the same as the relationship for patients within trials (20). This means that our findings do not exclude the possibility that a relation may be found within a high-risk population. Furthermore, our principal finding pertains to the different nature of the relationship across categories of risk rather than to the presence of a relation per se. Results from the Framingham Heart study indicated a graded inverse relation of FMD with Framingham risk score in subjects without prevalent cardiovascular disease (28). The Framingham risk was presented in quintiles rather than in absolute scores, and age was not used in the calculation of the Framingham risk. Because this population comprises healthy community volunteers, it might be that the finding in the paper by Benjamin et al. (28) reflects the left part of Figure 1, (i.e., subjects with relatively low risk). If so, the graded association of FMD with Framingham risk agrees with our hypothesis.

Flow-mediated dilation is currently used in an increasing number of studies as a measure of endothelial function. It has been suggested that FMD may help in risk stratification, and the utility of FMD measurements in randomized controlled trials as primary outcome as an alternative for cardiovascular events has been suggested. The latter notion is based on the evidence that endothelial dysfunction is related to unfavorable levels of cardiovascular risk factors, to presence of vascular damage, and to future risk of cardiovascular disease. Moreover, it is assumed that FMD is an equally accurate measure of endothelial function both in healthy individuals and in patients with advanced atherosclerosis. Although we did not study whether endothelial dysfunction predicts future cardiovascular events, the findings may indicate that, indeed, in high-risk patients, the measured FMD might not reflect endothelial function completely.

In conclusion, our analyses show that, only in populations at low risk, endothelial function measured by FMD is related to the principal cardiovascular risk factors, and to the estimated 10-year risk of CHD.

Reprint requests and correspondence: Dr. Michiel L. Bots, Julius Center for Health Sciences and Primary Care, Stratenum 6.131., University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands. E-mail: M.L.Bots@umcutrecht.nl.

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APPENDIX

For a list of the meta-analysis references, please see the June 21, 2005, issue of *JACC* at www.onlinejacc.org.