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# Flow-Mediated Dilation and Cardiovascular Event Prediction

## Does Nitric Oxide Matter?

Daniel J. Green, Helen Jones, Dick Thijssen, N.T. Cable, Greg Atkinson

**Abstract**—Endothelial dysfunction is an early atherosclerotic event that precedes clinical symptoms and may also render established plaque vulnerable to rupture. Noninvasive assessment of endothelial function is commonly undertaken using the flow-mediated dilation (FMD) technique. Some studies indicate that FMD possesses independent prognostic value to predict future cardiovascular events that may exceed that associated with traditional risk factor assessment. It has been assumed that this association is related to the proposal that FMD provides an index of endothelium-derived nitric oxide (NO) function. Interestingly, placement of the occlusion cuff during the FMD procedure alters the shear stress stimulus and NO dependency of the resulting dilation: cuff placement distal to the imaged artery leads to a largely NO-mediated response, whereas proximal cuff placement leads to dilation which is less NO dependent. We used this physiological observation and the knowledge that prognostic studies have used both approaches to examine whether the prognostic capacity of FMD is related to its role as a putative index of NO function. In a meta-analysis of 14 studies (>8300 subjects), we found that FMD derived using a proximal cuff was at least as predictive as that derived using distal cuff placement, despite the latter being more NO dependent. This suggests that, whilst FMD is strongly predictive of future cardiovascular events, this may not solely be related to its assumed NO dependency. Although this finding should be confirmed with more and larger studies, we suggest that any direct measure of vascular (endothelial) function may provide independent prognostic information in humans. (*Hypertension*. 2011;57:363-369.)

**Key Words:** flow-mediated dilation ■ methodology ■ cuff placement ■ NO ■ predictive value

### Why Consider Flow-Mediated Dilation a Prognostic Index?

Cost-effective prevention of cardiovascular disease (CVD) is predicated on cardiovascular risk assessment, which aims to distinguish between individuals at high and low risk of future disease manifestation. An optimal screening method should be inexpensive, relatively noninvasive and reproducible. It should predict risk in patients with known disease and, ideally, the future risk of events in currently healthy asymptomatic populations.<sup>1</sup>

Despite several decades of development and refinement, algorithms for the prediction of cardiovascular risk in humans that are based on “traditional” or “conventional” risk factors fail to predict a substantial proportion of cardiovascular events.<sup>1</sup> A recent review pertaining to identification of the “vulnerable patient” concluded that high Framingham risk scores fall short in accurately predicting events in individual patients and cannot provide a clear clinical route for identification or treatment of near future victims of acute coronary syndrome or sudden death.<sup>1</sup> A recent computed tomography angiographic study of 1650 subjects concluded that only 55%

of patients with a high plaque burden had high National Cholesterol Education Program/Framingham risk scores. Of the patients identified as high risk by Framingham in this study, 10% had no evidence of plaque, 32% with no plaque were taking statins based on traditional risk assessment, and 21% of those defined as requiring statins based on established computed tomography presence of plaque were unmedicated based on their risk factor profile.<sup>2</sup> It was concluded that Framingham and National Cholesterol Education Program risk categories do not reflect the presence of atherosclerotic disease.

There are several possible reasons for this risk factor prediction “gap.” Epidemiology-derived models based on the prediction of long-term risk may not adequately determine short-term events in individual patients, whereas emerging and novel risk factors (eg, physical inactivity/fitness) are often not included in the models. Perhaps more importantly, ≈70% of acute myocardial infarction and/or sudden coronary death are because of plaque rupture in patients with no previous history of being at high risk for atherosclerotic disease.<sup>3</sup> Indeed, the factors that predispose plaque to rupture

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**Table 1. Studies Investigating FMD as a Predictor of Prognosis in Subjects With CVD or at High Risk for CVD**

First Author	Journal	N	Male, %	Age, y	Group	Follow-Up	End Points (n)	Prognostic Impact
Meyer	<i>J Am Coll Cardiol</i>	75	89	56	CHF	1.5	Progression	FMD predicts progression disease (independent)
Neunteufl	<i>Am J Cardiol</i>	73	52	51	Chest pain+CAG	5	CV events (23)	FMD predicts CV events (independent)
Gokce	<i>J Am Coll Cardiol</i>	199	82	67	PAD	1.2	CV events (35)	FMD predicts CV events (independent)
Fathi	<i>J Am Coll Cardiol</i>	444	59	59	CAD	2	CV events (119)	Low risk: FMD does not predict CV events; high risk: FMD predicts CV events (independent)
Brevetti	<i>Circulation</i>	131	90	64	PAD	1.9	CV events (39)	FMD predicts CV events (independent)
Karatzis	<i>Am J Cardiol</i>	98	100	63	NSTEMI	2	CV events (20)	FMD predicts CV events (independent)
Fischer	<i>European Heart Journal</i>	67	61	82	CHF	3.9	CV events (24)	FMD predicts CV events (independent)
Chan	<i>J Am Coll Cardiol</i>	152	56	84	CAD	2.8	CV events (22)	FMD/GTN ratio predicts CV events (independent)
Frick	<i>J Am Coll Cardiol</i>	398	100	54	Chest pain+CAG	3.3	CV events (44)	FMD does not predict CV events
Patti	<i>Circulation</i>	136	81	63	CAD+stent	0.5	Restenosis (20)	FMD predicts restenosis
Kitta	<i>J Am Coll Cardiol</i>	141	68	66	PCI	0.5	Restenosis (46)	FMD (during follow-up) predicts restenosis

CV indicates cardiovascular; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CAG, coronary angiography; CHF, congestive heart failure; NSTEMI, non-ST elevation myocardial infarction; PAD, peripheral artery disease; GTN, glyceryl trinitrate.

are not necessarily the same as the risk factors that cause atherosclerotic build-up, and it has been argued recently that prediction of cardiovascular events might be improved if it included factors that render patients vulnerable to plaque rupture and thrombus formation.<sup>1,3</sup> Vulnerability of plaque has been related to factors such as acute inflammation, a thin cap, history of fissure, presence of calcified nodes, intraplaque hemorrhage, and outward remodeling.<sup>1,3</sup> Importantly, endothelial denudation or dysfunction is also a defining criterion for plaque vulnerability. Because endothelial dysfunction is also a final common pathway or “barometer” of the combined impact of traditional atherosclerotic risk factors,<sup>4</sup> assessment of endothelial (dys)function in humans presents an attractive option for determination of risk associated with plaque vulnerability, thrombogenesis, and combined risk factor impact on atherogenesis.

### Does Flow-Mediated Vasodilation Provide Clinically Relevant Prognostic Information?

The proposal that flow-mediated vasodilation (FMD) may provide useful prognostic information is based on the concept that direct assessment of the function of the arterial wall, rather than measurement of risk factors that impact on it, may improve predictive power. Table 1 summarizes the results of studies that have assessed FMD as a prognostic index in patients with CVD or at high risk for incident CVD. FMD independently predicted cardiac events in the majority of these studies, collectively involving ≈2000 subjects. In addition, FMD independently predicts restenosis in patients who receive bare-metal or drug-eluting stents.<sup>5,6</sup> These data strongly support the predictive value

of measuring FMD in patients at moderate to high risk of CVD and suggest that FMD provides independent prognostic information that may exceed that available from traditional risk factors in such subjects.

Table 2 summarizes studies undertaken to determine the independent (using various degrees of covariate adjustments) prognostic role of FMD in a total of ≈10 000 asymptomatic subjects. Results from these studies have been conflicting, with some supporting the independent prognostic value of FMD,<sup>7,8</sup> whereas others suggest no greater (no less) predictive capacity relative to risk factor assessment.<sup>9,10</sup> In one of the largest studies,<sup>10</sup> FMD was an independent predictor but added little to the best predictive model involving traditional risk factors. Because this study was performed in an aged cohort (72 to 98 years), it has been suggested that FMD may become less predictive in older individuals in whom arterial distensibility is limited.<sup>11</sup> Indeed, a more recent study from this group in younger subjects (61 years)<sup>12</sup> indicated that FMD was an independent predictor of CVD events and that this inverse association remained significant after adjustment for multiple CVD risk factors and the Framingham risk score. In general, the studies summarized in Table 2 suggest that FMD is predictive of cardiovascular events and provides prognostic information that is at least as predictive as that available from measurement and combination of numerous traditional risk factors.

### Is Repeated Measurement of FMD a Better Prognostic Option?

FMD reflects dynamic vascular homeostasis and can be modified by interventions including drugs, dietary intake,<sup>13</sup>

**Table 2. Studies Investigating FMD as a Predictor of Prognosis for Future CVD in Asymptomatic Humans**

First Author	Journal	N	Male, %	Age, y	Group	Follow-Up	End Points (n)	Prognostic Impact
Shechter	<i>Int J Cardiol</i>	435	65	54	Asymptomatic	2.7	CVevents (48)	FMD predictor for CV events (independent)
Rossi	<i>J Am Coll Cardiol</i>	2264	0	54	Asymptomatic	3.8	CVevents (90)	FMD predictor for CV events (independent)
Shimbo	<i>Atherosclerosis</i>	842	42	67	Asymptomatic	3	CVevents (30)	FMD predictor for CV events (not independent)
Yeboah	<i>Circulation</i> (2009)	3026	50	61	Asymptomatic	5	CVevents (198)	FMD predictor for CV events (not independent)
Yeboah	<i>Circulation</i> (2007)	2792	41	79	Asymptomatic	5	CVevents (674)	FMD predicts events, but does not add to current models

CV indicates cardiovascular.

and exercise.<sup>14</sup> It is also likely that variability in FMD is lower within subjects than between individuals. The dynamic nature of FMD raises the possibility of adopting a treat-to-target approach, where FMD can be intermittently monitored with the goal of normalizing or enhancing vascular health. A number of studies have assessed whether change in FMD provides important prognostic information in humans (Table 3). These studies indicate that improvement in FMD identifies those patients with more favorable prognosis and those in whom persistent impairment despite optimization of therapy is a significant independent predictor of events. Interestingly, some of these studies failed to detect significant relationships between baseline FMD measures and subsequent events. This suggests that changes in FMD may be more valuable in risk stratifying than relying on a single FMD measurement at a given point in time.

Taken together, the studies described above provide clear evidence for the prognostic relevance of FMD. This was confirmed by a recent meta-analysis,<sup>15</sup> which suggested a 13% (95% CI: 9% to 17%) decrease in the future risk of cardiovascular events exists for every 1% increase in FMD.

### Physiological Basis for the Prognostic Value of FMD

A principal physiological rationale for the association between FMD and cardiovascular prognosis is the assumption that it reflects nitric oxide (NO) bioavailability. The physiological

assumptions underlying the FMD technique were reviewed recently in detail.<sup>16</sup> Endothelium-derived NO possesses myriad antiatherogenic and plaque stabilizing properties, including regulation of vascular tone and arterial wall stress, inhibition of cell growth and proliferation, leukocyte and platelet adhesion, and antithrombotic and fibrinolytic properties.<sup>17,18</sup>

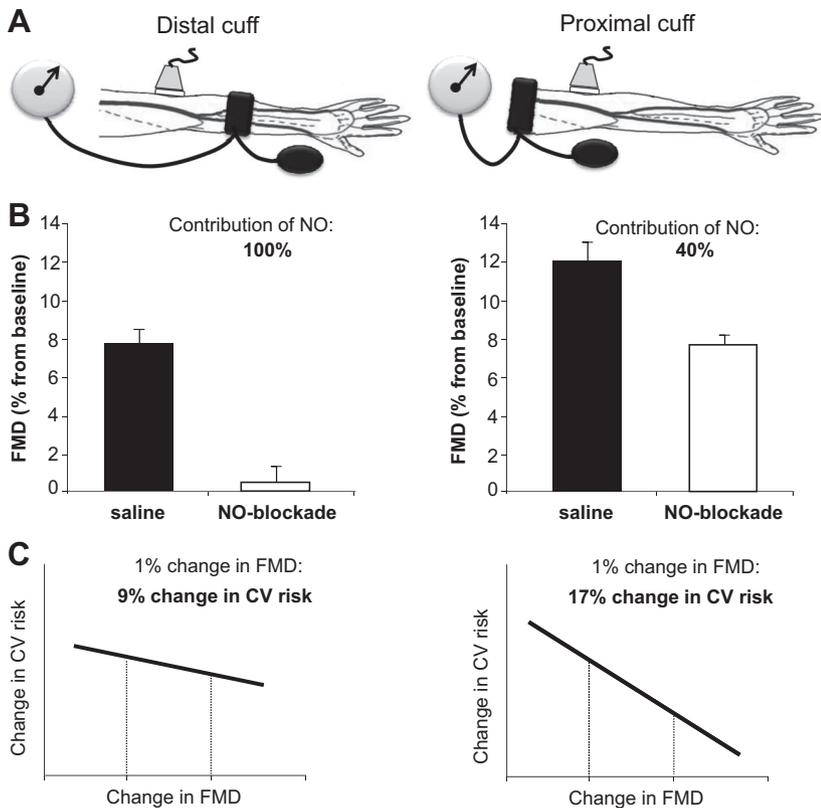
Rubanyi et al<sup>19</sup> and Pohl et al<sup>20</sup> suggested that the substance released from the endothelium in response to flow, such as that occurring after cuff-induced ischemia (FMD), possessed the characteristics of endothelium-derived relaxing factor, NO,<sup>21</sup> and in situ studies using NO antagonists decrease FMD.<sup>22,23</sup> Subsequent animal studies consolidated the link between increases in flow, wall shear stress, endothelial NO synthase expression, and NO bioactivity.<sup>24,25</sup> Together, this evidence strongly suggests that flow and shear are physiological stimuli that induce NO production from the endothelium in vivo. However, it is important to note that other vasoactive substances can also be released by the endothelium in response to shear stress (eg, prostacyclin and endothelial derived hyperpolarizing factor).<sup>26–28</sup>

Although Celermajer et al<sup>29</sup> provided no direct evidence that their particular FMD technique induced dilation that could be blocked by NO antagonists, subsequent studies suggested that dilation resulting from cuff occlusion can be NO dependent. For example, radial artery FMD (3.6%), after 3 minutes of ischemia induced by a wrist cuff placed distal to

**Table 3. Studies Assessing the Impact of Change in FMD as a Predictor of Prognosis for Future CVD in Humans**

First Author	Journal	N	Male, %	Age, y	Group	Follow-Up	End Points (n)	Prognostic Impact
Kitta	<i>J Am Coll Cardiol</i>	251	58	67	CAD	3	42	Repeated FMD, but not single FMD, predicts restenosis
Kitta	<i>J Am Coll Cardiol</i>	141	68	66	PCI	0.5	Restenosis (46)	Repeated FMD, but not single FMD, predicts restenosis
Suessenbacher	<i>Vasc Med</i>	68	n/a	56	Post-PCI	1.2+3.5	10	Repeated FMD, but not single FMD, predicts CV events
Fichtlscherer	<i>Circulation</i>	198	83	55	ACS	0.2	11	Repeated test predicts repeat ACS
Chan	<i>J Am Coll Cardiol</i>	106	n/a	n/a	CAD	2.8	8	Change in FMD predicts CV events
Modena	<i>J Am Coll Cardiol</i>	400	0	57	Hypertension	5.5	47	Repeated FMD has larger prognostic value than single FMD

CV indicates cardiovascular; n/a, not applicable; CAD, coronary artery disease; PCI, percutaneous coronary intervention; ACS, acute coronary syndromes.



**Figure. A,** Assessment of FMD using an occluding cuff placed distal (left) or proximal (right) to the imaged artery. **B,** Adapted from Doshi et al,<sup>39</sup> depicts the NO dependency of FMD under each cuff placement condition, as determined by upstream infusion of either saline (control) or the NO-blocker  $N^G$ -monomethyl-L-arginine. FMD is highly NO dependent when the artery lies upstream from the occluding cuff (left) but less NO dependent if the imaged artery lies within the ischemic territory during occlusion (right). **C,** Prognostic value of FMD, presented as the change in risk for a future cardiovascular event associated with a change of 1% in the FMD value. Proximal cuff placement, although less NO dependent, is nonetheless more predictive of CV events, challenging the orthodoxy that FMD owes its prognostic capacity to its assessment of NO bioavailability.

the ultrasound probe was converted to constriction ( $-2.8\%$ ) in the presence of NO blockade,<sup>30</sup> whereas brachial infusion of  $N^G$ -monomethyl-L-arginine decreased the radial artery FMD response to 5 minutes of distal wrist cuff occlusion from  $\approx 5.3\%$  to  $0.7\%$ ,<sup>31</sup> with no difference in the hyperemic stimulus in the control versus  $N^G$ -monomethyl-L-arginine conditions. A more recent study also indicated that femoral artery FMD, induced by a 5-minute cuff occlusion period, was NO dependent.<sup>32</sup> Although not a universal finding,<sup>33</sup> these studies<sup>30–32</sup> and others<sup>34</sup> suggest that FMD is NO dependent in humans and that reductions in the shear stress stimulus magnitude during NO blockade are not responsible for the FMD attenuation.

However, not all of the protocols leading to an FMD response in humans are equally NO dependent.<sup>35–37</sup> A number of researchers have suggested that FMD protocols that induce shear stress responses of greater magnitude than those associated with the approach by Celermajer et al<sup>29</sup> are less NO dependent. For example, the radial artery FMD response to 5 minutes of distal wrist cuff occlusion was largely NO mediated, whereas 15 minutes of cuff inflation induced higher shear stress and FMD ( $9.6\%$ ), which was not affected by NO blockade ( $9.6\%$  versus  $9.5\%$ ).<sup>31</sup> Other studies indicate that cuff placement is important. Placement of the occluding cuff above the imaged artery results in a larger shear rate stimulus and a larger dilation of the conduit artery<sup>38,39</sup> but also has an impact on the mechanisms responsible for the dilation.<sup>39</sup> In the study by Doshi et al<sup>39</sup> a 5-minute cuff occlusion at the wrist, distal to the ultrasound probe placed on the brachial artery, was associated with an  $\approx 7\%$  FMD response, which was abolished by  $N^G$ -monomethyl-L-

arginine infusion, whereas cuff placement on the arm above the ultrasound probe resulted in  $\approx 12\%$  FMD, which was only partially decreased by NO blockade (Figure). These and other findings strongly suggest that interventions that induce shear stress of larger magnitude are associated with the release of multiple vasoactive substances that contribute to conduit artery dilation, whereas the controlled  $\approx 5$ -minute stimulus of Celermajer et al<sup>29</sup> is more NO dependent. That the former tests remain endothelium dependent, if not largely NO mediated, is assumed on the basis that arterial dilation in response to increased flow/shear depends on the presence of an intact endothelial layer in animals<sup>20,25</sup> and humans.<sup>40,41</sup>

### Does FMD Predict Cardiovascular Events Because It Is a Surrogate for NO Bioactivity?

It is a feature of the FMD prognosis studies presented in the tables that different measurement methods have been adopted (Table 4). Many studies adopted a method involving upper arm cuff placement, whereas others have used distal limb ischemia. As described above, both approaches will induce FMD which is endothelium dependent, but the distal limb method is evidently more NO dependent. To address the question of whether FMD is a strong prognostic index because it reflects NO function, we reanalyzed the recent meta-analysis data provided by Inaba et al<sup>15</sup> by assessing the prognostic strength of those studies that used distal versus proximal cuff placement (Table 4). Full details regarding study inclusion and exclusion criteria can be found in the original meta-analysis.<sup>15</sup> Using the online Supplementary Data (available at <http://hyper.ahajournals.org>) or information elicited from direct contact with the authors, we obtained

**Table 4. Details of Studies That Were Included in the Calculation of the Prognostic Role of the FMD for Cardiovascular Events,<sup>15</sup> With Cuff Placement Proximal or Distal (Shaded Region) From the Imaged Artery**

First Author	Year	N	Male, %	Age, y	Group	Follow-Up, y	Annual Mortality, %	Cuff Position	Occlusion, min	RR per 1% FMD ↑
Shimbo	2007	842	42	67	Asymptomatic	3	0.19	Proximal	5	0.94 (0.84 to 1.04)
Frick	2005	398	100	54	Chest pain+CAG	3.3	0.3	Proximal	5	0.93 (0.82 to 1.05)
Meyer	2005	75	89	56	CHF	1.5	12	Proximal	5	0.85 (0.74 to 0.99)
Shechter	2009	435	75	54	Asymptomatic	2.7	0.2	Proximal	5	0.83 (0.72 to 0.96)
Neunteufl	2000	73	52	51	Chest pain+CAG	5	0	Proximal	4.5	0.77 (0.67 to 0.90)
Gokce	2003	199	82	67	PAD	1.2	2	Proximal	5	0.77 (0.69 to 0.86)
Patti	2005	136	81	63	CAD+stent	0.5	0	Proximal	5	0.76 (0.69 to 0.85)
Fathi	2004	444	59	59	CAD	2	5.5	Distal	4.5	0.98 (0.94 to 1.01)
Yeboah	2007	2792	41	79	Asymptomatic	5	2.4	Distal	4	0.96 (0.93 to 1.00)
Rossi	2008	2264	0	53	Asymptomatic	3.8	0.03	Distal	5	0.89 (0.84 to 0.95)
Brevetti	2003	131	90	64	PAD	1.9	1.2	Distal	5	0.87 (0.78 to 0.97)
Karatzis	2006	98	100	63	NSTEMI	2	1.9	Distal	4.5	0.86 (0.75 to 0.98)
Muiesan	2008	172	59	57	Hypertension	7.9	0.2	Distal	5	0.85 (0.76 to 0.96)
Katz	2005	259	84	54	CHF	2.3	2	Distal	5	0.83 (0.70 to 0.99)

CAG, coronary angiography; CAD, coronary artery disease; PAD, peripheral artery disease; CHF, congestive heart failure; NSTEMI, non-ST elevation myocardial infarction; RR, relative risk.

the risk ratios and methodologic information from the 14 studies that were included in the meta-analysis. Inaba et al<sup>15</sup> calculated a multivariate-adjusted risk ratio, for each study, that represented the decrease in risk for every 1% increase in FMD. We coded each study in terms of the classic technique by Celermajer et al,<sup>29</sup> involving distal cuff placement and FMD, which is NO dependent (7 studies; 6160 participants), or in terms of a proximal cuff localization and FMD, which is endothelium dependent but may not be as NO mediated (7 studies; 2158 participants). For studies in which a distal cuff placement was selected, we calculated that a 1% increase in FMD was associated with a relative risk of 0.91, that is, a 9% (95% CI: 4% to 13%) decrease in the future risk of cardiovascular events. By comparison, studies involving proximal cuff localization revealed a relative risk of 0.83, that is, a 17% (95% CI: 12% to 22%) decrease in cardiovascular risk for every 1% increase in FMD. Using an unrestricted maximum likelihood mixed-effects metaregression approach with distal and proximal placement coded as a dummy variable,<sup>42</sup> the difference between these 2 relative risks was found to be statistically significant ( $P=0.01$ ).

We undertook a number of sensitivity analyses to verify that other factors (eg, study quality and sample size) identified by Inaba et al<sup>15</sup> to moderate these risk ratios were not different between the 2 cuff-placement study cohorts. First, we reanalyzed the data after removal of 2 atypical studies by Gocke et al<sup>43</sup> and Fathi et al,<sup>44</sup> which may have exhibited abnormally high rates of events, because the former involved patients with peripheral artery disease and FMD measurements obtained immediately after surgery, and the latter study included ≈40% of the participants who were undergoing hemodialysis when FMD was measured. When these 2 studies were removed, the relative risk per percentage of increase in FMD was 0.89 (95% CI: 0.84 to 0.94) for distal and 0.85 proximal (95% CI: 0.79 to 0.90). The difference between these relative risks approached statistical signifi-

cance ( $P=0.10$ ) and was in the direction of greater prediction from proximal cuff placement.

Two probably related moderators could have been study size and type of population, with the general population-based studies being somewhat larger than those involving clinical symptomatic patients. We therefore undertook sensitivity analysis involving the studies on symptomatic patients only. We found relative risks per percentage increases in FMD of 0.89 (95% CI: 0.83 to 0.96) and 0.81 (95% CI: 0.74 to 0.88) for distal and proximal cuff placement, respectively ( $P=0.03$ ). When the studies on population-based asymptomatic people were analyzed, the relative risk per percentage increases in FMD were 0.93 (95% CI: 0.86 to 0.99) and 0.89 (95% CI: 0.79 to 0.99) for distal and proximal cuff placement, respectively ( $P=0.50$ ). We then subdivided the studies according to whether they involved large (>200 participants) or small (<200 participants) sample sizes. Although the cohort of small studies still showed a significant difference ( $P=0.04$ ) in relative risk per percentage increase in FMD between distal (0.86 [95% CI: 0.80 to 0.91]) and proximal (0.78 [95% CI: 0.73 to 0.83]), the analysis of the larger studies, per se, did not show such a difference ( $P=0.31$ ). We found that the mean study quality, as rated by Inaba et al,<sup>15</sup> was similar between distal (18.0 U) and proximal cohorts (17.3 U). Therefore, although our results are robust to the influence of study population and some study-specific clinical interventions, we cannot rule out that study size and some specific clinical aspects of individual studies may have influenced our findings. Nonetheless, it is relevant to note that, in all cases, distal cuff placement was not superior to proximal cuff placement in terms of predictive capacity.

Somewhat surprisingly then, it seems that endothelium-dependent FMD, which is purportedly less NO dependent, may be more predictive, or at least equally predictive, of cardiovascular events. This raises several questions. Has the importance of endothelium-derived NO been exaggerated? Is

an endothelium-dependent stimulus that elicits the production of a range of vasodilators more prognostically relevant than one that is more unimodal? On the latter question, there is already evidence that measures such as the blood flow or shear stress response after cuff release, indicators of microvascular function,<sup>45,46</sup> possess independent predictive value,<sup>47,48</sup> and such responses are not NO mediated. Ultimately, answers to these questions will require data from a prospective randomized trial of different stimuli derived from the same subjects.

## Summary

Prediction of cardiovascular risk has traditionally involved assessment of risk factors, but this approach is relatively ineffective. Endothelial dysfunction is an early atherogenic event and is also associated with decreased vulnerability of plaque to rupture. Measurement of endothelial (dys)function has, therefore, been proposed as a useful adjunct to cardiovascular risk factor assessment and studies relating FMD to future risk show strong prognostic relevance. It has generally been assumed that this association is related to the proposal that FMD provides an index of NO-mediated vasodilator function and is, therefore, a marker of in vivo NO bioavailability. However, we provide evidence that prognostic studies that used an FMD approach that is less NO dependent may be at least as predictive of events. Although this does not diminish findings that FMD exhibits strong prognostic power, however measured, it does suggest that this may not be solely because of NO. We, therefore, propose that any direct measure of vascular (endothelial) function may provide independent prognostic information in humans, which complements that available from traditional risk factor assessment. This proposal is broadly in keeping with recent suggestions that traditional cardiovascular risk factor-based prediction models, such as the Framingham score, are improved by the addition of direct assessments of arterial imaging.<sup>49</sup>

Finally, we agree with Inaba et al<sup>15</sup> that the presence of heterogeneity in study quality, confounding factors, and publication bias in the available literature prevent a definitive evaluation of the additional predictive value of brachial FMD beyond traditional cardiovascular risk factors, and we also acknowledge that future research confirming the present results pertaining to NO dependency of the predictive value of FMD is necessary before the use of proximal occlusion can be considered a superior method of assessment compared with that currently adopted.<sup>16</sup>

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## Disclosures

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