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Heterogenous Nature of Flow-Mediated Dilatation in Human Conduit Arteries In Vivo

Relevance to Endothelial Dysfunction in Hypercholesterolemia

Michael J. Mullen,* Rajesh K. Kharbanda,* Jenny Cross, Ann E. Donald, Mia Taylor, Patrick Vallance, John E. Deanfield, Raymond J. MacAllister

Abstract—Flow-mediated dilatation (FMD) of conduit arteries is dependent on an intact endothelium, although the mechanisms are not fully understood. Using high-resolution ultrasound, we examined the role of endothelial mediators in radial artery dilatation in response to transient (short period of reactive hyperemia) and sustained (prolonged period of reactive hyperemia, hand warming, or an incremental infusion of acetylcholine into the distal radial artery) hyperemia. After short episodes of reactive hyperemia, FMD was abolished by local infusion of the nitric oxide synthesis inhibitor *N*^Gmonomethyl-L-arginine ($5.3 \pm 1.2\%$ versus $0.7 \pm 0.7\%$, $P < 0.001$). In contrast, basal vessel diameter and dilatation after prolonged episodes of reactive hyperemia, hand warming, and distal infusion of acetylcholine were not attenuated by nitric oxide synthesis inhibition. Inhibition of cyclooxygenase or local autonomic nervous system blockade also had no effect on FMD. Patients with hypercholesterolemia exhibited reduced FMD in response to transient hyperemia, but the response to sustained hyperemia was normal. These data suggest heterogeneity of endothelial responses to blood flow that are dependent on the characteristics of the flow stimulus. Dilatation after brief episodes of hyperemia is mediated by release of nitric oxide, whereas dilatation during sustained hyperemia is unaffected by NO synthesis inhibition. Hypercholesterolemia seems to differentially affect these pathways with impairment of the nitric oxide-dependent pathway and preservation of non nitric oxide-mediated dilatation to sustained flow stimuli. (*Circ Res.* 2001;88:145-151.)

Key Words: endothelium ■ nitric oxide ■ flow-mediated dilatation ■ vascular physiology

Conduit arteries dilate in response to an increase in blood flow.¹⁻⁴ This physiological response is dependent on the presence of an intact endothelium,^{5,6} and the measurement of flow-mediated dilatation (FMD) in vivo has been widely adopted as an assessment of endothelial function.^{7,8} Abnormalities of FMD have been demonstrated in patients with clinical coronary artery disease² and in younger, preclinical subjects with risk factors for atherosclerosis.⁷

Endothelial cells are sensitive to shear stress and respond by synthesizing factors that regulate vascular smooth muscle tone.^{9,10} Endothelium-derived vasodilators that have been identified include nitric oxide (NO) and prostacyclin, and the existence of an endothelium-dependent hyperpolarizing factor has been proposed.¹¹ In humans, dilatation of conduit arteries in response to reactive hyperemia is reduced by inhibitors of NO synthesis, suggesting an important role for NO in FMD.^{12,13} However, several other studies have suggested that under different physiological conditions, FMD occurs by mechanisms that are independent of NO production. In animals¹⁴ and hu-

mans,¹⁵ coronary FMD in response to sustained hyperemia induced by distal infusion of adenosine seems resistant to the effects of inhibition of NO synthesis. These data suggest heterogeneity of the endothelial response to blood flow, whereby the physical characteristics of the flow stimulus might be important in determining the mechanism of the subsequent dilatation.¹⁶ Characterizing the biology of these responses in humans has important implications for understanding the regulation of vascular tone and interpreting the results of endothelial function tests in risk-factor groups.

In this study, we examined the relationship between conduit artery blood flow and dilatation to determine how the dynamic characteristics of the flow stimulus influence the mechanisms of conduit artery dilatation in healthy humans and patients at risk for atherosclerosis.

Materials and Methods

Healthy volunteers 18 to 45 years of age were recruited from staff members. Patients with hypercholesterolemia were recruited from outpatient clinics and asked to stop cholesterol-lowering medication

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for 2 weeks before being studied. No subjects had any history of cardiovascular disease, and all were nonsmokers, nondiabetic, and taking no vasoactive medication. Studies were approved by the local research ethics committee, and all subjects gave written informed consent.

Measurement of Conduit Artery Diameter and Blood Flow

Experiments were carried out in a temperature-controlled laboratory ($23 \pm 1^\circ\text{C}$). High-resolution ultrasound was used to image the brachial or radial artery of the nondominant arm. The vessel was scanned in longitudinal section using an Acuson XP10 ultrasound system and a 10-MHz linear-array transducer supported by a stereotactic clamp. The image was magnified using a resolution box function and gated with the R wave of the ECG. Fine adjustments in the position of the transducer were made by means of micrometer screws attached to the base of the clamp to maintain image quality throughout the study. Sequential end-diastolic images of the artery were acquired every 3 to 5 seconds throughout each study using data acquisition software (Information Integrity), and the diameter of a 1- to 2-cm segment was determined for each image using a semiautomatic edge-detection algorithm.¹⁷ Blood flow at the same site as where vessel diameter was being measured was recorded continuously throughout the study using pulsed wave Doppler. Systemic blood pressure was measured in the contralateral arm at regular intervals throughout each study using an automated sphygmomanometer (Dinamap).

Assessment of the Role of Vasoactive Mediators in Radial Artery FMD

Radial artery blood flow was manipulated using several methods developed in our laboratory and dilatation in response to different hyperemic stimuli assessed in the absence and presence of inhibitors of potential endothelial pathways. The brachial or radial artery was cannulated at the antecubital fossa under local anesthesia using a 27-gauge needle. Vehicle (0.9% saline) or drugs (see below) dissolved in vehicle were infused intra-arterially at 0.5 mL/min. All pharmacological inhibitors were preinfused for at least 10 minutes before assessing their effect on FMD.

Protocol 1

Reactive hyperemia was induced by inflation of a distal pneumatic cuff to 300 mm Hg for 5 minutes followed by its deflation (Figure 1A). Radial artery FMD in response to this brief blood-flow stimulus was measured before and after intra-arterial infusion of the NO synthase inhibitor *N*^Gmonomethyl-L-arginine (L-NMMA) ($4 \mu\text{mol}/\text{min}$; preinfused for 10 minutes; $n=8$) or administration of the cyclooxygenase inhibitor aspirin (600 mg orally, $n=6$).

Protocol 2

Sequential 5- and 15-minute cuff inflations were used to induce brief and more sustained episodes of reactive hyperemia. Radial artery dilatation in response to these stimuli was assessed before and after L-NMMA, as described above ($n=8$).

Protocol 3

Hand warming (from 22°C to 45°C) was used to cause a sustained increase in radial artery blood flow (Figure 1B). FMD in response to this sustained flow stimulus was assessed during intra-arterial infusion of saline followed by infusion of L-NMMA ($4 \mu\text{mol}/\text{min}$, $n=8$, and $16 \mu\text{mol}/\text{min}$, $n=5$) and L-NMMA ($4 \mu\text{mol}/\text{min}$) together with aspirin ($10 \text{ mg}/\text{min}$, $n=7$) and during local autonomic blockade (intra-arterial infusion of atropine $350 \text{ ng}/\text{min}$, propranolol $40 \mu\text{g}/\text{min}$, and phentolamine $25 \mu\text{g}/\text{min}$; $n=7$). Dilatation in response to hand warming was also assessed before and 2 hours after administration of aspirin (1200 mg orally, $n=6$).

Protocol 4

A stepwise increase in sustained blood flow was induced in the radial artery by an incremental infusion of acetylcholine (10, 100, 500, and 1000 nmol/min; each dose for 5 minutes, $n=6$) into the radial artery

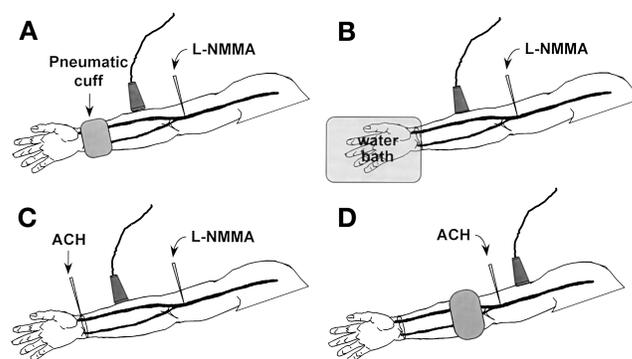


Figure 1. Methods of stimulating increased blood flow. Blood flow and vessel diameter were measured using vascular ultrasound, and the effect of pharmacological inhibitors on flow-mediated responses was determined from the actions of infused drugs via the brachial artery (proximal to the segment of artery being studied). A, Inflation of the wrist cuff for 5 or 15 minutes resulted in reactive hyperemia of differing duration in the proximal radial artery. B, Warming of the hand to 42°C to 45°C caused sustained hyperemia in the radial artery. C, Infusion of an incremental dose of acetylcholine into the radial artery at the wrist was used to increase blood flow to the hand and cause a stepwise sustained increase in radial artery blood flow. D, Inflation of a forearm cuff for 5 minutes or infusion of acetylcholine into the brachial artery was used to increase brachial artery blood flow.

at the wrist (Figure 1C). Radial artery blood flow and dilatation in response to this stimulus were assessed during infusion of saline followed by L-NMMA ($4 \mu\text{mol}/\text{min}$) via the brachial artery.

Assessment of Effect of Hypercholesterolemia on FMD

Protocol 5

Brachial artery FMD in response to transient (reactive hyperemia, induced by a 5-minute cuff occlusion) and sustained (incremental infusion of acetylcholine at doses of 10, 100, and 1000 nmol/min; each dose for 5 minutes) hyperemia was assessed in 9 hypercholesterolemic subjects and 9 control subjects (Figure 1D).

Drugs

The following drugs were used: acetylcholine (Clinalpha), aspirin (Laboratories Synthelabo), atropine (Antigen Pharmaceuticals Ltd), lignocaine (Antigen Pharmaceuticals Ltd), L-NMMA (Clinalpha), phentolamine (Ciba), propranolol (Zeneca Pharma), and 0.9% saline (Baxter Healthcare Ltd).

Data Analysis

Blood flow was expressed as the velocity time integral (VTI) (the area under the blood velocity/time curve for a complete cardiac cycle). VTI (m) was determined at baseline (mean of at least 2 measurements during the first minute of each study) and at prespecified time points (every 5 seconds for the first 15 seconds and then every 15 seconds during reactive hyperemia and every minute during steady-state hyperemia) during hyperemia. Baseline vessel diameter (mm) was defined as the mean of all measurements during the first minute of each study. Dilatation (maximal after reactive hyperemia and mean of 1 minute during steady-state conditions) was expressed as a percentage change from the baseline diameter. The time course of blood flow velocity or dilatation was analyzed by measuring the area under the time curve (AUC).

Statistical Analysis

All results are expressed as mean \pm SE and compared using Student's *t* test for paired or unpaired observations as appropriate or by ANOVA. Values of $P < 0.05$ were considered significant.

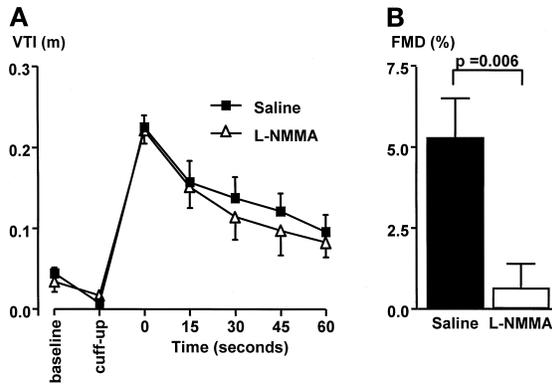


Figure 2. L-NMMA had no effect on radial artery blood flow at baseline or during cuff inflation or reactive hyperemia (A) but significantly attenuated FMD in response to reactive hyperemia (B).

Results

None of the interventions to alter blood flow in the radial artery had any effect on systemic hemodynamics, as assessed by heart rate and blood pressure or flow and radial artery dilatation in the contralateral arm (data not shown).

Mechanism of Radial Artery Dilatation After Reactive Hyperemia

Inflation of the occluding cuff almost completely abolished radial artery blood flow and was associated with a significant reduction in radial artery diameter of $-3.9 \pm 1.1\%$. After a 5-minute period of distal forearm ischemia, peak VTI was maximal within 15 seconds (0.23 ± 0.01 m), but it returned to baseline by 1 minute. In response to this stimulus, radial artery diameter increased by $5.3 \pm 1.2\%$ (maximal at 69.3 ± 8.1 seconds after release of the cuff, AUC 438 ± 163 U). L-NMMA had no effect on radial artery VTI at rest during cuff inflation or reactive hyperemia (Figure 2A). Similarly, L-NMMA had no significant effect on arterial constriction observed during cuff inflation but attenuated FMD (maximal dilatation $0.7 \pm 0.7\%$ $P=0.006$, AUC -251 ± 102 U $P<0.001$; Figure 2B). Aspirin had no significant effect on resting radial artery blood flow or FMD in response to a 5-minute cuff occlusion ($4.7 \pm 0.5\%$ versus $4.9 \pm 0.5\%$, $P=NS$).

In a separate study, we compared the effects of L-NMMA on FMD in response to reactive hyperemia after 5 and 15 minutes of wrist cuff occlusion. After the 15-minute cuff occlusion, peak VTI was not significantly increased compared with the standard 5-minute cuff occlusion (0.2 ± 0.05 versus 0.25 ± 0.04 , $P=0.05$), but the duration of reactive hyperemia was prolonged, resulting in a significant increase in the AUC of blood flow (17.5 ± 3.5 versus 11.7 ± 3.5 U, $P=0.006$; Figure 3A). This resulted in a significantly enhanced maximal radial artery dilatation from $5.4 \pm 0.7\%$ to $9.6 \pm 0.7\%$ ($P<0.001$; Figure 3B) and AUC of the dilatation/time response curve from 522 ± 102 to 1374 ± 162 U. ($P<0.001$). L-NMMA attenuated maximal FMD after the 5-minute cuff occlusion (2 ± 0.6 , $P=0.02$) but, in contrast, had no effect on maximal radial artery dilatation after the

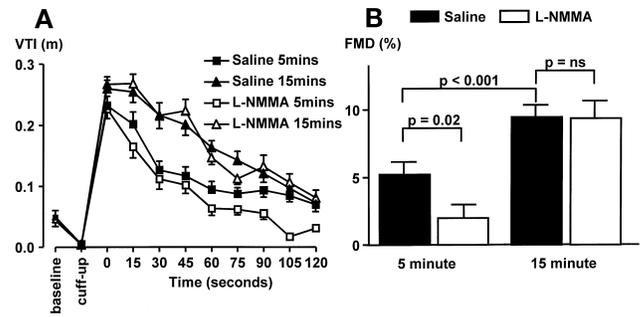


Figure 3. Fifteen minutes of hand ischemia resulted in a more prolonged episode of reactive hyperemia (A) and a significantly increased flow-mediated dilatation of the radial artery (B) compared with 5 minutes of ischemia. L-NMMA had no effect on reactive hyperemia. L-NMMA reduced FMD in response to 5 minutes of ischemia but had no effect on FMD in response to more sustained flow caused by the longer period of hand ischemia.

15-minute cuff occlusion ($9.6 \pm 0.7\%$ versus $9.5 \pm 1.1\%$, $P=0.8$; Figure 3B) or the AUC of the time/dilatation response curve (1254 ± 186 U during L-NMMA, $P=0.4$).

Mechanism of FMD in Response to Sustained Hyperemia

Hand warming increased radial artery blood flow velocity from 0.02 ± 0.01 to 0.11 ± 0.01 m. Despite the relatively low peak blood-flow velocity achieved compared with that produced by reactive hyperemia, this sustained stimulus resulted in a mean radial artery dilatation of $9.7 \pm 1.8\%$. Infusion of L-NMMA ($4 \mu\text{mol}/\text{min}$) had no significant effect on radial artery blood flow or dilatation to this sustained flow stimulus (dilatation 11.2 ± 2.8 ; Figure 4). An additional 10-minute infusion of L-NMMA at $16 \mu\text{mol}/\text{min}$ to compensate for the dilutional effect of the increase in blood flow also had no effect on dilatation associated with hand warming (dilatation $10.2 \pm 2\%$). Inhibition of cyclooxygenase with oral aspirin had no significant effect on blood flow or radial artery dilatation after hand warming ($8 \pm 1.9\%$ versus $7.42 \pm 3.5\%$; $P=0.7$; $n=6$). Intra-arterial aspirin ($10 \text{ mg}/\text{min}$) infused with L-NMMA ($4 \mu\text{mol}/\text{min}$) also had no effect on radial dilata-

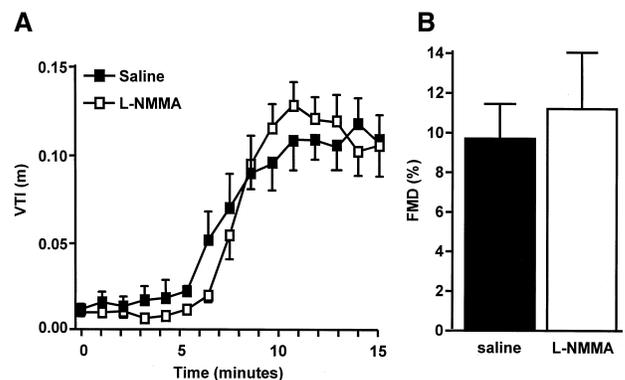


Figure 4. Hand warming caused a gradual and sustained increase in blood flow in the radial artery (A), which was associated with a contemporaneous radial artery vasodilatation (B). L-NMMA had no effect on hyperemia or FMD.

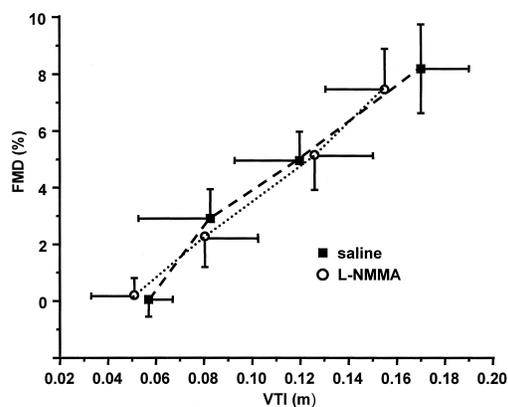


Figure 5. An incremental infusion of acetylcholine via the radial artery resulted in a graded increase in blood flow (VTI). This was associated with a stepwise dilatation of the proximal radial artery that was quantitatively and temporally related to the increase in blood flow. Infusion of L-NMMA had no effect on the relationship between radial artery blood flow and dilatation.

tion during hand warming ($9.1 \pm 1.6\%$ versus $9.8 \pm 2.6\%$; $P=0.7$; $n=7$).

Local autonomic blockade abolished the constrictor response of the radial artery during the cold pressor test ($n=3$; data not shown) but had no significant effect on radial artery blood flow in response to hand warming or the consequent radial artery dilatation (FMD $8.3 \pm 1.8\%$ before and $10.9 \pm 1.8\%$ after autonomic blockade; $P=0.1$; $n=7$).

Infusion of acetylcholine into the distal radial artery caused a dose-dependent increase in blood flow that was associated with radial artery dilatation upstream from the site of infusion. Infusion of L-NMMA into the brachial artery significantly reduced blood flow in response to acetylcholine (necessitating higher doses to produce the same blood flow) but had no effect on radial artery dilatation in response to equivalent flow stimuli (Figure 5).

Characterization of FMD in Response to Transient and Sustained Flow Increases in Patients With Hypercholesterolemia

There were significant differences in total and LDL cholesterol levels between the hypercholesterolemic and control groups, but other parameters were comparable (Table). There were no significant differences in resting brachial artery blood flow (0.058 ± 0.01 versus 0.075 ± 0.014 , $P=0.32$) or diameter (4.11 ± 0.16 versus 4.45 ± 0.28 , $P=0.34$) and the

Subject Characteristics

	Control	Hypercholesterolemic	<i>P</i>
No. (% male)	9 (78)	9 (78)	
Age, y	33 ± 1.7	38 ± 1.7	0.08
Total cholesterol, mmol/L	4.35 ± 0.28	6.96 ± 0.45	<0.001
LDL cholesterol, mmol/L	2.30 ± 0.21	4.81 ± 0.51	<0.001
HDL cholesterol, mmol/L	1.75 ± 0.17	1.66 ± 0.13	0.69
Triglyceride, mmol/L	0.66 ± 0.08	1.03 ± 0.09	0.01

Data are mean \pm SE. Groups were compared using independent Student's *t* tests.

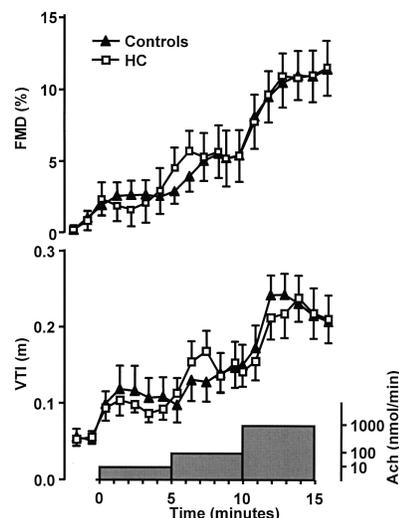


Figure 6. Infusion of an incremental dose of acetylcholine into the forearm resulted in a dose-dependent, steady-state increase in brachial artery blood flow in both control subjects and subjects with hypercholesterolemia. This was associated with a stepwise dilatation of the brachial artery. In contrast to the response to reactive hyperemia, this was not different in subjects with hypercholesterolemia compared with control subjects. ACH indicates acetylcholine; HC, hypercholesterolemia.

degree of reactive hyperemia induced by 5-minute forearm cuff inflation (AUC 12.61 ± 1.72 versus 12.47 ± 1.23 , $P=0.95$). However, brachial artery dilatation in response to reactive hyperemia was significantly lower in hypercholesterolemic subjects compared with control subjects ($4.6 \pm 0.6\%$ versus $7.2 \pm 0.7\%$, $P=0.04$).

Distal infusion of acetylcholine increased brachial artery blood flow in a dose-dependent manner, which was similar in both groups and resulted in an equivalent stepwise dilatation of the brachial artery in both groups (Figure 6). There was no difference in the slopes of the flow/dilatation response curves between hypercholesterolemic subjects and control subjects, and on ANOVA, brachial artery dilatation was significantly associated with blood flow ($P=0.001$) but not the presence of hypercholesterolemia or cholesterol level.

Discussion

This study demonstrates that under normal physiological conditions, different types of flow stimulus elicit different mechanisms of conduit artery dilatation in humans. Dilatation, in response to transient increases in blood flow, is largely mediated by synthesis of NO, whereas sustained dilatation during a prolonged hyperemic stimulus is unaffected by L-NMMA, indicating an NO-independent mechanism. Furthermore, in patients with hypercholesterolemia, these pathways were differentially affected, with a selective abnormality only of the NO-dependent component. Our findings indicate that the mechanisms of conduit artery FMD in vivo are more complex than previously thought and have important implications for the design and interpretation of endothelial function tests and the treatment of vascular dysfunction in cardiovascular disease.

In the present study, pharmacological blockade of physiological pathways was used to probe the mechanisms that regulate radial artery diameter under different blood flow conditions. Previous reports have demonstrated a role for NO in radial artery dilatation in response to reactive hyperemia.^{12,13} This finding was confirmed in the present study in which FMD after a brief episode of reactive hyperemia was almost completely abolished during infusion of L-NMMA, an effect that was not explained by any change in the flow stimulus. In contrast, L-NMMA did not significantly alter basal radial artery diameter or the dilator response to a sustained flow stimulus caused by a prolonged episode of reactive hyperemia, local hand warming, or an incremental infusion of acetylcholine into the hand. The dose of L-NMMA used has previously been shown to cause near maximal inhibition of NO-mediated dilatation,¹⁸ and increasing the dose of L-NMMA 4-fold during hand warming did not alter the response.

We considered the possibility that nonflow-related mechanisms, such as local ischemia, vasoactive metabolites, or neuronal mechanisms, might mediate dilatation during sustained flow. We developed several different methods for inducing a sustained flow stimulus that were unlikely to have identical collateral effects to cause endothelium-independent dilatation. We observed a close temporal association between changes in blood flow and arterial diameter during these protocols. Similarly, when radial artery blood flow was maintained at basal levels during hand warming, there was no significant dilatation of the radial artery. In addition, using distal infusion of acetylcholine at incremental doses allowed us to establish a clear relationship between sustained flow and dilatation over a range of different flow intensities. There were no effects of any flow stimulus on systemic hemodynamics, blood flow, or dilatation in the contralateral radial artery. These observations strongly suggest that the brachial and radial artery dilatation we observed was a direct consequence of changes in luminal blood flow. L-NMMA had no effect on dilatation to sustained flow regardless of the method used to induce hyperemia, and, therefore, we conclude that under these conditions, NO-independent mechanisms mediate arterial dilatation in response to a sustained flow stimulus. The effects of L-NMMA on radial artery tone under resting conditions support these findings. Reduced blood flow during cuff inflation resulted in significant radial artery constriction, implying the presence of tonic flow-mediated dilatation (in response to normal resting sustained flow conditions). However, in the 31 studies where L-NMMA was infused into the radial artery, no constriction was apparent. Although it is possible that our methods did not detect a small effect of L-NMMA, these data suggest that such basal FMD was to a greater degree independent of NO synthesis.

The absence of an effect of L-NMMA or aspirin on resting flow and arterial diameter is not consistent with previous reports of reduced forearm blood flow during NO and prostaglandin synthesis inhibition measured using plethysmography.^{18,19} However, we assessed radial artery physiology in the mid forearm, which subtends only a relatively small muscle bed in the hand. Similarly, neither L-NMMA, aspirin, nor autonomic blockade had a significant effect on

radial artery blood flow during reactive hyperemia or hand warming, and we were able to compensate for the effect of L-NMMA on hyperemia induced by acetylcholine by using a higher dose. Thus we were able to examine the effects of these agents on FMD under similar conditions of hyperemia. This might explain the difference in our results from those of previous studies in the coronary circulation, in which significant reduction in epicardial coronary artery diameter was demonstrated at rest and during hyperemia after NO synthesis inhibition^{15,20–24} and aspirin.²³ In the majority of these studies, the pharmacological agent has had a major effect on coronary blood flow, and thus it is difficult to separate the effect of direct pathway inhibition from the effect of reduced blood flow.

The mechanism of dilatation in response to sustained flow is presently unclear but might involve endothelial- or nonendothelial-dependent pathways. We found no effect of aspirin on dilatation in response to any of the flow stimuli used, suggesting a minimal role for vasoactive prostanoids in FMD and in accord with previous studies.^{12,25} Similarly, pharmacological blockade of the autonomic nervous system had no effect on radial artery FMD in response to hand warming, consistent with animal studies showing that FMD is preserved after surgical or pharmacological denervation.^{26,27} Alternative mechanisms that we have not tested might involve the release of endothelium-derived hyperpolarizing factors, activation of potassium channels, or stimulation of sensory nerves. It is also possible that under physiological conditions, NO contributes to conduit artery dilatation in response to a sustained flow stimulus, but that during reduced NO synthesis, alternative mechanisms compensate.^{28,29} Combined infusion of L-NMMA and aspirin did not affect dilatation in this study, excluding the possibility of interaction between the NO and prostaglandin pathways, but additional experiments with blockade of multiple pathways will be needed to test this hypothesis.

These findings suggest that a physiological role of the NO pathway is to provide a mechanism to limit the degree to which shear stress is elevated in response to rapid changes in blood flow and imply that there is adaptation of the response of the NO pathway. Whether this occurs because of reduced NO production or desensitization to the effects of NO is unclear, but understanding how the pathway habituates might have implications for understanding how activity of the NO pathway is reduced in cardiovascular disease. If the NO pathway has a similar role in resistance vessels, then it might provide a mechanism for the rapid buffering of variations in blood flow and pressure. Consistent with this hypothesis is the observation that inhibition of NO production in experimental animals and humans increases blood pressure variability.^{30–32}

Consistent with our previous report,³³ FMD after a brief period of reactive hyperemia was significantly impaired in patients with hypercholesterolemia compared with normocholesterolemic control subjects. In contrast, however, no abnormality of dilatation in response to a sustained flow stimulus was apparent. We chose to use a distal infusion of acetylcholine as the stimulus in this patient group to investigate flow-mediated dilatation in response to sus-

tained flow over a range of flow intensities, because we believed that this would have the greatest sensitivity for detecting a difference in the response to sustained flow between the 2 groups. These data suggest a selective abnormality of the NO pathway in hypercholesterolemia with relative preservation of non NO-mediated responses. It is possible that this abnormality reflects impaired sensing or transduction of the flow stimulus. However, previous reports of impaired agonist-mediated NO synthesis in hypercholesterolemia^{34,35} would favor an effect distal to flow-transduction mechanisms. Additional experiments will be required to determine whether FMD in response to sustained flow is preserved in other risk factor groups and patients with established cardiovascular disease.

These data are relevant to the design and interpretation of endothelial function tests that use flow as a stimulus. The noninvasive measurement of FMD in peripheral conduit arteries is a method that has been developed in our laboratory⁷ and has been widely adopted as an *in vivo* assessment of endothelial function. We and others have previously demonstrated abnormalities of FMD in association with cardiovascular risk factors from an early age⁷ and its restoration after intervention.^{36,37} Correct interpretation of these studies, however, will depend on a detailed knowledge of the physiology of FMD and the mechanisms that underlie the interactions with risk factors. Abnormalities of FMD in response to brief periods of reactive hyperemia are likely to reflect reduced NO bioactivity. In contrast, dilatation after the use of more intense flow stimuli (eg, after placing the occluding cuff on the upper arm or using prolonged periods of ischemia) might be largely determined by NO-independent mechanisms, and these may not be effected by risk factors for atherosclerosis.

In this study, we have demonstrated that the mechanisms of conduit artery dilatation to flow in humans *in vivo* are heterogeneous and determined by the physical and dynamic characteristics of the flow stimulus. Our data indicate that the role of NO in regulating conduit artery tone may be more limited than previously thought, with the maintenance of arterial dilatation under basal conditions or during sustained hyperemia being largely mediated by NO-independent mechanisms. Impaired FMD in risk-factor groups such as hypercholesterolemia might represent a specific abnormality of the NO-dependent pathway, with relative preservation of FMD in response to sustained flow.

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