Are the dynamic response characteristics of brachial artery flow-mediated dilation sensitive to the magnitude of increase in shear stimulus?

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Pyke KE, Hartnett JA, Tschakovsky ME. Are the dynamic response characteristics of brachial artery flow-mediated dilation sensitive to the magnitude of increase in shear stimulus? J Appl Physiol 105: 282–292, 2008. First published May 8, 2008; doi:10.1152/japplphysiol.01190.2007.—The purpose of this study was to determine the dynamic characteristics of brachial artery dilation in response to step increases in shear stress [flow-mediated dilation (FMD)]. Brachial artery diameter (BAD) and mean blood velocity (MBV) (Doppler ultrasound) were obtained in 15 healthy subjects. Step increases in MBV at two shear stimulus magnitudes were investigated: large (L; maximal MBV attainable), and small (S; MBV at 50% of the large step). Increase in shear rate (estimate of phase I to total FMD when two phases occurred was not sensitive to shear rate magnitude (slope $r^2 = 0.003$, slope $P = 0.775$). Parameters quantifying the dynamics of the FMD response [time delay (TD), time constant ($\tau$)] were also not sensitive to shear rate magnitude for both phases (phase I: TD $r^2 = 0.03$, slope $P = 0.376$, $\tau r^2 = 0.04$, slope $P = 0.261$; final phase: TD $r^2 = 0.07$, slope $P = 0.169$, $\tau r^2 = 0.07$, slope $P = 0.996$). These data support the existence of two distinct mechanisms, or sets of mechanisms, in the human conduit artery FMD response that are proportionally sensitive to shear stimulus magnitude and whose dynamic response is not sensitive to shear stimulus magnitude.

Doppler ultrasound; hyperemia; endothelium

The proper function of the endothelial cells that line the arteries is essential for vasoregulation and vascular health. In a healthy artery, an increase in blood flow-associated shear stress results in an endothelial-dependent, flow-mediated dilation (FMD) (21, 24, 28). As a result, FMD, in response to an increase in shear stress, can serve as an index of endothelial function and, therefore, is thought to be a biosassay of vascular health. Originally created by Celermajer et al. (7), the test most commonly performed in humans creates a large transient increase in shear stress in a conduit artery (usually the brachial or radial) via reactive hyperemia following the release of a temporary limb occlusion. When assessed with this reactive hyperemia test, individuals with atherosclerosis or with risk factors for the development of cardiovascular disease have reduced FMD compared with healthy controls (6, 8, 10, 12, 17). The early discovery of this correlation has resulted in a paucity of studies investigating other stimulus profiles, including more sustained stimuli. However, longer, more moderate increases in shear stress represent arguably more physiologically relevant stimuli in that they more closely approximate the normal in vivo experience than the large, brief stimulus of reactive hyperemia.

We have created a technique that uses forearm plus hand warming to reduce downstream vascular resistance, combined with brachial artery compression to permit the creation of controlled step increases in brachial artery shear stress. Unlike the transient stimulus created with reactive hyperemia, or even the gradual increase in shear stress created by heating without arterial compression (2, 15, 20), a step increase stimulus profile allows meaningful quantification of FMD response dynamics. This is because a step change creates a constant stimulus, and thus the response dynamics clearly reflect the action of the underlying mechanisms. If, in contrast, the stimulus is changing, the response dynamics also depend on the rate of change of the stimulus.

Response dynamics include the time delay (TD) between the onset of the stimulus and the initiation of the response, and the rate of increase quantified by the time constant ($\tau$) of the response (specifically the time to 63% of peak response development). Response dynamics provide more detailed information about the nature of control mechanisms than the peak response to a stimulus. For example, multiphased responses indicate that distinct mechanisms or sets of mechanisms are engaged sequentially over time. This can be seen in blood flow dynamics, where an initial, rapid increase plateaus within 5–7 s to be followed by a slower second phase, which begins ~20 s after exercise onset. This represents two distinct sets of mechanisms for arteriolar vasodilation (25). If only the peak response magnitude is measured, the presence of multiple phases cannot be detected. This is important because it is becoming increasingly appreciated that the mechanisms initiating FMD may be distinct from the mechanisms that maintain it (11, 20, 26). Characterization of the time course of the response also allows an assessment of dynamic linearity (16). If a system demonstrates dynamic linearity, the same rate of response development occurs, regardless of the magnitude of stimulus increase. Dynamic linearity suggests that the same control mechanisms are responsible for the adaptation to different stimulus magnitudes.

The purpose of this study was to provide the first characterization of human brachial artery FMD magnitude and time course in response to a sustained step increase in shear stress. The results demonstrate two distinct phases of FMD, which are proportional to the magnitude of the shear stimulus increase and possess dynamic response characteristics that are unaffected by the magnitude of the shear stimulus increase.

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METHODS

Subjects

Fifteen healthy, nonsmoking male subjects between the ages of 19 and 27 yr from the Queen’s University student population volunteered to participate. Health status of the subjects was confirmed with a medical screening questionnaire for risk factors associated with endothelial dysfunction. Each subject served as his own control. The study protocol was approved by the Health Sciences Human Research Ethics board at Queen’s University, and all subjects completed a consent form that was approved by the same board. Subjects were instructed to abstain from alcohol, caffeine, and exercise for 12 h before the study, and to abstain from food for 4 h before the study. Each subject performed both trials on each of 3 test days at the same time of day (±2 h) in a quiet, temperature-controlled room (24°C). Each of the 3 test days took place over a 10-day period, with the exception of one subject for whom the test days were spread over 15 days.

Subject monitoring. Heart rate (HR) was monitored throughout each study via three-lead ECG. Blood pressure was measured continuously via arterial tonometry (Colin 7000, Trudell Medical Institute, London, ON) or finger photoplethysmography (Finapres, Ohmeda).

Measurement of Brachial Artery Blood Velocity and Diameter

Brachial artery blood velocity was measured continuously in all experiments with Doppler ultrasound operating at 4 MHz (GE Vingmed System 5, GE Medical Systems). The Doppler shift frequency spectrum was analyzed via a Multigon 500V TCD (Multigon Industries) spectral analyzer from which mean velocity was determined as a weighted mean of the spectrum of Doppler shift frequencies. The corresponding voltage output was, in turn, continuously sampled at 200 Hz and stored (Powerlab, AD Instruments) for later analysis.

All scans were performed at an insonation angle of 68°. This angle was selected because it allows the vessel to be perpendicular to the ultrasound beam, and this yields superior image quality. Image quality was of primary concern, since error in measurement of diameter change as small as 0.1 mm can represent 2.5% FMD in a 4-mm brachial artery. This angle did not compromise validity and precision of blood velocity measurement, as described previously (22). Briefly, our Doppler was subject to the following careful calibration procedure. The ultrasound probe was positioned to insonate tygon tubing immersed in a water bath at an angle of 68°. Water with ultrasound reflecting particles (dissolved corn starch) was pumped through the tubing at a number of known flow rates representing actual mean flow velocities from <2 to >120 cm/s. The Doppler frequency spectrum analysis results in a continuous voltage output representing the instantaneous mean velocity of flow. This was plotted against the actual known velocity to provide a linear calibration slope. This linear relationship between voltage output and velocity was linear with $r^2 = 0.98$ and highly reproducible.

The ultrasound probe was oriented over the brachial artery to achieve a clear arterial blood velocity signal, with no interference from adjacent vein blood flow. Once in position, the probe was secured with a clamp stand and a guide adhered to the skin. The brachial artery was imaged by two-dimensional grayscale ultrasound imaging in B-mode with the same probe operating at 10 MHz. The probe operator was able to make minor corrections to probe placement to maintain an optimal velocity signal and vessel image throughout the experiment while maintaining a consistent insonation angle within subject trials. The images were recorded in Digital Imaging and Communications in Medicine format for future analysis with a custom-automated edge-detection software (29).

Experimental Procedures

Subjects lay supine with both arms out to their sides. Blood pressure was measured on the right arm, whereas ultrasound measurements were performed on the left arm.

Forearm heating. Up to the level of the anticubital fossa, the forearm was enclosed in a custom water bath (empty at baseline). The bath consists of a 6-in.-diameter tube with an internal plastic sleeve that covers the hand and forearm so that they are not in direct contact with the water. Warm water (maintained between 43 and 45°C) from an external water heater was then pumped in to fill the bath. Once full, recirculation was started such that the water was continually draining from the bath via gravity into the water heater to be reheated, and then pumped back into the bath (Fig. 1). Skin temperature was continuously monitored (Barnant thermistor thermometer 600-1070) and not allowed to rise above 42°C. Ongoing confirmation of the subject’s comfort level guided immediate adjustments in water temperature (infusion of cold water), if the subject began to experience discomfort.

Arterial compression. At the initiation of water bath filling, the subject’s brachial pulse was located just proximal to the anticubital fossa (distal to site of ultrasound measurement). A custom-designed stand equipped with a linear actuator (McMaster Carr), tipped with a domed stylus, was placed over the brachial pulse. Using custom-designed controlling software, the stylus was lowered to provide arterial compression. Manipulation of this compression allowed control of upstream blood velocity through the brachial artery, where increasing compression slowed down blood velocity and controlled release of compression allowed blood velocity to increase. Blood velocity was displayed online as a 5-s moving average, allowing the experimenter to adjust arterial compression to achieve and maintain a target blood velocity. Arterial compression did not result in an enhancement in retrograde velocity compared with uncompressed velocity recordings in either compression baseline or the large and small trials.

Experimental protocol. Figure 2 illustrates the experimental protocol. Baseline brachial artery images and blood velocity before filling the water bath were recorded for 1 min (precompression baseline). Blood velocity was monitored continuously throughout the protocol. Once filling of the water bath was initiated, arterial compression commenced, and blood velocity was maintained at ~3 cm/s throughout the heating protocol. To ensure a maximal reduction in forearm vascular resistance, heating and arterial compression were performed for 30 min before the first trial was started.

The first trial was the large step increase in blood velocity (and therefore shear rate). One minute of brachial artery diameter and blood velocity was recorded before the large step (compression baseline). The arterial compression was then fully released, and the averaged blood velocity observed in the first 15–20 s post-compression release became the target velocity. Arterial compression was used to control blood velocity at that target for 20 min. The arterial compression was then increased to bring blood velocity back down to ~3 cm/s until baseline diameter was reestablished (minimum of 10 min). The target for the small step increase in blood velocity was calculated as 50% of the increase that took place in the preceding large trial. One minute of baseline velocity and brachial artery diameter was recorded before controlled release of arterial compression (compression baseline). Like the large step trial, the small step duration was 20 min. The order of the trials was not counterbalanced (large trial always performed first), because quantification of the large trial shear was required to determine the appropriate small trial shear. Subjects underwent the same protocol (large step followed by small step) on each of the 3 test days. The three large and three small trials (one from each day) for each subject were averaged to provide one mean large step response and one mean small step response profile.

In all three trials for the first subject and two of three trials in the second subject, stimulus and response parameters were only recorded for 15 min. The missing last 5 min of data were, therefore, extrap-
lated from the exponential equation used to fit the first 15 min, as derived from our curve fitting procedure (see below).

Data Analysis

Brachial artery blood velocity. Blood velocity data were compiled as 3-s time bins for each trial, allowing multiple trials to be time aligned and averaged. This resulted in a single representative mean blood velocity profile for each subject.

Brachial artery diameter. Vessel diameter was analyzed using an updated version of automated edge-detection software (FMD/Blood Flow Acquisition and Analysis), described in Woodman et al. (29). This program allows the user to identify a region of interest on the portion of the image where the walls are most clear. It then identifies and tracks the walls of the artery via the intensity of the brightness of the walls vs. the lumen of the vessel. The program collects one diameter measurement for every pixel column in the region of interest. It uses the median diameter as the diameter for that frame. The program is triggered to the ECG signal and provides a diameter measurement for every R wave (corresponding to end diastole). The diameter data were compiled as 3-s time bins and combined similar to blood velocity. The three trials were averaged together to reduce noise and facilitate fitting an exponential function to the data (25). Missing data due to tracking error in individual trials were interpolated to facilitate calculation of the average. This mean profile was then plotted over time, and an exponential function line of best fit was determined using custom software as follows: initial parameter estimates (TD: time from onset of stimulus to onset of response; and τ: time to 63% of response magnitude) are entered for the appropriate number of phases evident in the data (1, 2, or 3 phases). The model applied allows for a separate TD for each component of the response (Fig. 3). The fitting program is then provided with a range for each parameter within which it performs 100 iterations, resulting in modified parameter estimates, which form the new starting point for the next series of 100 iterations. This process is repeated until the mean sum of squares of the residuals has been minimized. Using the finalized exponential function parameters, another custom program then calculated diameter values along that function in 3-s intervals. Thus a diameter measurement and a velocity measurement, time aligned for every 3 s, were obtained. FMD is reported as the percent change in diameter (%FMD) from the baseline measured before release of arterial compression (compression baseline).

Shear rate. Shear rate (an estimate of shear stress without viscosity) was calculated as mean blood velocity/vessel diameter and was used to quantify the stimulus for FMD. Shear rate was calculated from the 3-s average velocity bins, and the 3-s average diameter data were calculated from the line of best fit. The shear rate was then averaged.

Fig. 1. Schematic of the forearm heating setup. The subject’s arm is enclosed in a plastic sleeve that prevents direct contact with the water. The sleeve is sealed around the outside of the bath. Heated water circulates through the bath. Forearm heating dilates skin resistance vessels to create a potential for sustained increases in brachial artery blood velocity. Software-controlled movement of stylus imparts desired arterial compression to “recruit” this potential and regulate upstream brachial artery blood velocity.

Fig. 2. Protocol timeline. Flow velocity refers to brachial artery mean blood flow velocity.
into 21-s time bins. The shear stimulus is reported as the mean shear rate or the mean increase in shear rate from baseline during the 20-min arterial compression release period.

Statistical Analysis

To determine whether dynamic response characteristics of FMD were sensitive to shear stimulus magnitude, linear regression of each response parameter against shear rate was performed. Repeated-measures analyses of variance and paired t-tests were used to compare the stimulus and response parameters between large and small shear step conditions. The level for significance was set at $P < 0.05$, and significant differences for ANOVA were further assessed using Tukey’s post hoc tests. All statistics were calculated using Sigmastat.

RESULTS

HR and Mean Arterial Blood Pressure

HR and mean arterial blood pressure (MAP) values are displayed in Table 1. In both the large and small step trials, HR was statistically significantly higher during the compression baseline and the arterial compression release period than during the precompression baseline. The 30-min compression period resulted in an elevated MAP in both the large ($P = 0.04$) and small ($P = 0.026$) step conditions. Compression release in the large step trial resulted in MAP returning to levels similar to the precompression baseline. In contrast, MAP remained elevated in the small step trial ($P = 0.004$). However, the percent increase in HR was virtually identical to that of MAP, indicating that MAP was increased via cardiac output, rather than peripheral vasoconstriction (27).

Brachial Artery Shear Rate

Compression baseline shear rate was $8.0 \pm 1.9$ and $8.4 \pm 1.8$ s$^{-1}$ for the large and the small trials, respectively ($P = 0.992$). The average shear rate during the release period was $84.8 \pm 16.1$ s$^{-1}$ for the large step and $49.7 \pm 9.1$ s$^{-1}$ for the small step ($P < 0.001$). This represents an average increase in shear rate of $76.8 \pm 15.6$ and $41.4 \pm 8.7$ s$^{-1}$ for the large and the small trials, respectively ($P < 0.001$) (Fig. 4). The small step increase in shear rate was $53.9 \pm 3.0\%$ of the large step increase, which is very close to our target of 50%.

Large step. See Fig. 5, A and C. Despite our intention to maintain a stable shear rate, it declined slightly over time. The average shear rate in the first 5 min was $88.5 \pm 18.3$ vs. $81.4 \pm 14.5$ s$^{-1}$ in the last 5 min ($P < 0.001$). This decline in shear rate was due to a slight decline in velocity (average velocity in the first 5 min: $37.4 \pm 5.7$ cm/s vs. the last 5 min: $36.2 \pm 5.0$ cm/s; $P = 0.007$) combined with a $\approx 14\%$ increase in brachial artery diameter (see following section).

Figure 5A also reveals an initial overshoot in the shear rate. To assess the magnitude of the overshoot, we compared the shear rate in the first two 21-s time bins to the average shear rate during the initial phase (phase I) of vasodilation. The shear rate in the first 21-s time bin ($102.8 \pm 28.9$ s$^{-1}$) was larger than the average shear rate during phase I ($88.5 \pm 19.0$ s$^{-1}$, $P < 0.001$). The shear rate in the second time bin ($91.1 \pm 23.8$ s$^{-1}$) was not significantly different from the average shear rate during phase I ($P = 0.556$). The high shear rate values in the first time bin result from a combination of a mild overshoot in velocity immediately post-compression release ($\approx 3$ cm/s) and the smaller diameter before vasodilation.

Small step. See Fig. 5, B and D. A stable shear rate was maintained throughout the small trial (shear rate in the first 5 min: $50.2 \pm 8.9$ s$^{-1}$ vs. the last 5 min: $49.3 \pm 8.9$ s$^{-1}$; $P = 0.06$). This
is a result of a stable velocity (average velocity in the first 5 min: 20.4 ± 2.6 cm/s vs. average velocity in the last 5 min: 20.4 ± 2.5 cm/s; P = 0.809) and a more modest diameter adaptation (~6%) (see following section). There was also no statistically significant overshoot in the small trial (first bin: 54.1 ± 13.9 s⁻¹ vs. phase I average shear rate: 50.4 ± 8.84 s⁻¹; P = 0.076).

Baseline Diameter and FMD

Precompression baseline diameter was 3.9 ± 0.4 mm, and the compression baseline diameters were 3.9 ± 0.4 and 4.0 ± 0.4 mm for the large and small trials, respectively (P = 0.410). The observation of no significant difference between the precompression and the compression baseline diameters indicates that heating alone with no increase in shear rate did not induce a conducted vasodilation of the brachial artery. This agrees with previous results from our group (22). The peak percent change in diameter was 14.5 ± 3.8% for the large step trial and 5.7 ± 2.3% for the small trial (P < 0.001) (Fig. 6A).

Shear Stimulus FMD Response Relationship

There was a strong relationship between the increase in shear rate and the peak %FMD (Fig. 6A; r² = 0.71, P < 0.001), and the phase I %FMD (Fig. 6B; r² = 0.67, P < 0.001). There was a moderate relationship between the increase in shear rate and the %FMD attributable to the delayed FMD [delayed %FMD = (final phase peak diameter − phase I peak diameter)/phase I peak diameter * 100] (Fig. 6C; r² = 0.39, P < 0.001). Small trials where no final phase FMD was present (n = 3) were not included in the delayed dilation regression.

Figure 7 illustrates the relationship between shear rate and the phase I FMD as a percentage of peak FMD when a final phase occurred. Three of 15 subjects did not exhibit FMD beyond phase I in the small step trial, so these are not included in the regression. There was no relationship between phase I FMD as a percentage of peak FMD and shear rate magnitude (r² = 0.003, P = 0.775 for slope different from zero; large step 72.24 ± 5.22% vs. small step 77.51 ± 11.38%, P = 0.085, n = 12, as subjects who did not demonstrate a final phase of dilation in the small step trial were removed to facilitate the paired t-test comparison). Thus the relative contribution of phase I FMD to peak FMD was not dependent on shear rate magnitude when a final phase FMD was present.

Response Dynamics

The time course of FMD in response to step increases in shear rate is displayed in Fig. 8, and an individual sample fit is
shown in Fig. 9. Figure 9 demonstrates the inappropriateness of a monoexponential fit to the data and reveals the biphasic nature of the physiological response. In the large step trial, an initial phase of dilation was followed by a middle stage that was characterized in one of four ways: 1) a plateau (8 of 15 subjects); 2) a minor dilation followed by a plateau (2 of 15 subjects); 3) minor reconstriction (4 of 15 subjects); or 4) a continued dilation followed by a reconstriction (1 of 15 subjects). This middle stage was then followed by a final phase of dilation in 12 of 15 subjects. In some subjects, the diameter response did not reach a complete plateau in the 20-min measurement period. We did not extrapolate beyond our 20 min of collected data; therefore, our values may modestly underestimate the response magnitude that could be achieved if the stimulus were maintained for a longer duration.

Fig. 6. A: relationship between the increase in shear rate from baseline and the peak percent flow-mediated dilation (FMD) over the 20-min stimulus period. B: relationship between the increase in shear rate from baseline and the phase I %FMD. C: relationship between the increase in shear rate from baseline and the delayed %FMD [delayed %FMD = (final phase peak diameter – phase I peak diameter)/phase I peak diameter × 100]. For all panels: open circles, small step; solid circles, large step; open square, small shear step mean; shaded square, large step mean. Error bars represent ± between-subjects SD.

Fig. 7. Relationship between the increase in shear rate from baseline and the phase I FMD as a percentage of peak FMD. This indicates the relative contribution of the phase I FMD to peak, across a range of shear rates. Open circles, small step; solid circles, large step; open square, small shear step mean; shaded square, large step mean (NS). Error bars represent ± between-subjects SD.

Fig. 8. A: average FMD response profile for small (open circles) and large (solid circles) shear step increase. Error bars represent the between-subjects SD. B: the dynamic response described by the mean of the individually fit kinetic parameters (τ and TD) for the large (solid line) and the small (dashed line) shear step increase.
The TD for phase I FMD (Fig. 10A) was not dependent on shear rate magnitude ($r^2 = 0.03, P = 0.370$ for slope different from zero). The group mean phase I TD for the small vs. large step was also not different ($P = 0.270$). Although the group mean TD for the final-phase FMD was significantly shorter in the large trial vs. the small trial ($P = 0.002, n = 12$), final-phase TD was not dependent on shear rate magnitude across the range of shear rates in this study ($r^2 = 0.07, P = 0.169$ for slope different from zero) (Fig. 10B). The overall average TD (large and small trials pooled) was $20.44 \pm 9.25$ s for phase I and $363.32 \pm 201.79$ s for the final phase.

The $\tau$ for phase I FMD (Fig. 10C) was not dependent on shear rate magnitude ($r^2 = 0.04, P = 0.261$ for slope different from zero). However, when analyzed as the group mean $\tau$ for the small vs. large shear step, it was close to reaching a statistically significant difference ($P = 0.054$). Close examination of the individual data revealed that there are two subjects whose $\tau$ value is $\pm 2$ SD away from the mean for the small trial. Statistical analysis within the remaining 13 subjects indicates that there was no significant difference in the $\tau$ for phase I FMD ($22.6 \pm 4.0$ vs. $26.7 \pm 10.4$ s $P = 0.193$). The overall average phase I $\tau$ was $27.91 \pm 13.99$ s.

The $\tau$ for final phase FMD (Fig. 10D) was not dependent on shear rate magnitude ($r^2 = 0.07, P = 0.995$ for slope different from zero), and, although the mean response magnitude was almost double in the large vs. small trials, it failed to reach statistical significance (large $818.5 \pm 849.1$ s vs. small $440.2 \pm 683.9$ s, $P = 0.237, n = 12$). The overall average final phase $\tau$ was $730.83 \pm 968.45$ s.

**DISCUSSION**

This study used a novel approach to control the magnitude and duration of a step increase in brachial artery shear stimulus. It provides the first characterization of human conduit artery FMD response dynamics in young, healthy humans. The primary novel findings are as follows. First, conduit artery FMD followed a generally biphasic pattern, with a fast initial phase (phase I) of dilation followed by a delayed, slower final phase of dilation. This is consistent with the existence of two
separate mechanisms or sets of mechanisms in the human brachial artery FMD response to a sustained increase in shear stimulus. Second, the relative contribution of the phase I FMD to peak FMD was insensitive to shear rate magnitude. Third, parameters quantifying dynamic response characteristics (TD, $\tau$) were insensitive to shear rate magnitude, providing the first evidence that FMD control mechanisms exhibit dynamic linearity (within the range of shear step magnitudes investigated). Finally, the magnitude of the peak FMD and phase I FMD was strongly related to the increase in shear rate, whereas the delayed FMD was moderately related. Thus both initiating and sustaining mechanisms of FMD appear to be proportionally sensitive to the magnitude of a step increase in shear stimulus.

**Dependency of FMD Response Magnitude on Shear Rate**

To date, methodological approaches to investigate FMD in human conduit arteries have focused on the assessment of the amplitude of the response to a transient reactive hyperemia stimulus (3, 4, 7, 8, 18) or distal heating-evoked gradual increases in shear stimulus to steady state (2, 15, 20). These groups reported a positive relationship between the sustained shear stress stimulus magnitude and the FMD response (2, 15, 20). Our findings are in agreement with this (see Fig. 6A).

However, our study extends these previous findings in three ways, because we were able to create step increases in shear rate. First, we demonstrate that the magnitude of the phase I FMD is also strongly associated with the shear stimulus magnitude (see Fig. 6B). Second, the magnitude of the delayed FMD is also impacted by shear stimulus magnitude, although this effect is not as strong (see Fig. 6C). Finally, we were able to partition the relative contribution of the phase I FMD to the peak FMD response and found that it was unaffected by shear rate magnitude. Taken together, these findings suggest that the mechanisms responsible for the initiation of FMD (phase I) are more strongly associated with shear stimulus magnitude than those determining the delayed FMD. Furthermore, the impact of shear stimulus magnitude on total FMD is predominantly mediated by phase I mechanisms across a wide range of shear rate increases.

**Is the Dynamic Response of FMD Dependent on the Magnitude of Shear Stimulus?**

Animal investigations of the time course of dilation in response to a sustained shear stimulus include conflicting reports of maintained (rat gastrocnemius and soleus arterioles) (26), transient (rat coronary arteries) (1), and biphasic...
Temporal Nature of FMD Mechanisms

As with the temporal characteristics of FMD, there exists considerable variability in the timing and magnitude of different shear-induced vasodilator activity. For example, observations in rat hindlimb skeletal muscle arterioles have indicated that the initial FMD magnitude (up to 2 min) is not dependent on nitric oxide (NO), while sustained FMD is completely NO dependent (26). In contrast, in isolated rat coronary arteries, NO inhibition resulted in a 50% reduction in the initial peak FMD magnitude and significantly increased the transient nature of the response (1). In rat gastrocnemius and soleus arterioles, Ca^{2+}-activated K^{+} channel blockade [endothelial derived hyperpolarizing factor (EDHF) pathway] reduced initial FMD magnitude (up to 2 min) by 69 and 44%, respectively (26), while it abolished the response in rat coronary arteries (1).

Finally, in human umbilical vein endothelial cells, Frangos et al. (11) found that G-protein inhibition eliminated an initial burst of NO production, while it had no effect on sustained NO production. Taken together, this lends further support to the hypothesis that the initial change in shear stress (step up) and the maintenance of that shear stress elicit distinct responses from the endothelium (11), and that this can be vessel and vascular bed specific.

Initial human studies utilizing transient reactive hyperemia and gradual shear increase with downstream heating-induced vasodilation found that FMD, in response to a brief reactive hyperemia shear stress stimulus, was mediated by NO, but that NO was not obligatory for FMD during a sustained shear elevation (20). Recent work by Bellien et al. (2) demonstrating a 40% reduction in sustained shear FMD with NO blockade contradicts these findings. These investigators also identified a potential obligatory role for EDHF in sustained shear FMD, whereby blockade reduced FMD by 15%. A NO-EDHF synergy was also evidenced by a 70% reduction in FMD with combined blockade (2).

The underlying basis for conflicting results regarding the role of NO in sustained shear FMD is unclear. One explanation that has not been considered is the potential for interindividual variability in the relative contribution of multiple FMD mechanisms. Thus, in the group of subjects studied by Mullen et al. (20), it may be that EDHF was able to completely compensate for the lack of NO production under N^{G}-monomethyl-L-arginine infusion. This type of compensatory response has been reported previously (14, 19).

Collectively, these previous animal and human results indicate the existence of distinct FMD initiating and maintaining mechanisms. This is in support of the current study’s interpretation of the biphasic nature of the response. Due to the conflicting findings regarding human steady-state sustained FMD, it remains unclear what mechanisms are obligatory. Further studies are necessary to resolve these conflicting results and examine the time course of involvement of the distinct mechanisms.

Potential Limitations

The experiments performed in this study were noninvasive. Therefore, comments concerning the underlying mechanisms based on the response dynamics are speculative. Future human studies involving pharmacological blockade are necessary to confirm the identity and time course of involvement of endothelial factors over time.

In the large shear step trial, the delayed onset of FMD, in combination with the mild overshoot in target velocity at arterial compression release, resulted in a shear rate with an initial overshoot (Fig. 5A). It is possible that the initial overshoot in shear rate affected the phase I FMD. To explore this, we assessed the relationship between the early overshoot in shear rate and the phase I \( \tau \) (\( r^{2} = 0.041; P = 0.467 \)) and the phase I TD (\( r^{2} = 0.058; P = 0.387 \)). The lack of any relationship argues strongly against the contention that the overshoot had an effect on the response dynamics. Furthermore, we recently demonstrated that the magnitude of initial peak reactive hyperemia-induced shear does not determine FMD (23).
There was a modest (~6–8%) increase in both HR and MAP during heating and arterial compression. This raises the possibility that elevated sympathetic activity directed at the brachial artery may have interfered with the FMD response. However, in addition to the modest magnitude of the change, two lines of evidence suggest that this is unlikely: 1) Even substantial elevations in sympathetic activation have not consistently been shown to blunt FMD (9). 2) The magnitude of the MAP change was matched by the magnitude of the HR change, and, therefore, the increase in MAP was likely mediated by an increase in cardiac output rather than peripheral vasoconstriction (27).

Finally, in this study, the magnitude of the large step increase in shear rate determined the target magnitude for the small step trial. As a result, the large trial had to be performed first, implicating a potential order effect. Arguing against this, however, there was no difference in the dynamics of the response between the two trials. Furthermore, while no previous studies have examined the effect of repeated exposure to sustained hyperemia, it has been demonstrated that repeated exposure to reactive hyperemia has no impact on the peak FMD (13). There is, therefore, no direct evidence to indicate the existence of an order effect; however, it cannot be completely ruled out with this data set.

Conclusion

This study provides the first characterization of the time course of human conduit artery FMD in response to a sustained shear stimulus. The biphasic nature of the response indicates the existence of two temporally distinct FMD mechanisms or sets of mechanisms in the human brachial artery. Both exhibit dynamic linearity and are proportionally sensitive to the magnitude of a step increase in shear, although initiating mechanisms appear predominantly responsible for the peak FMD being proportional to the shear stimulus.

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REFERENCES

6. Cellermaijer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with...

