Brachial artery flow-mediated dilation during handgrip exercise: evidence for endothelial transduction of the mean shear stimulus

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Pyke KE, Poitras V, Tschakovsky ME. Brachial artery flow-mediated dilation during handgrip exercise: evidence for endothelial transduction of the mean shear stimulus. Am J Physiol Heart Circ Physiol 294: H2669–H2679, 2008. First published April 11, 2008; doi:10.1152/ajpheart.01372.2007.—Exercise elevates shear stress in the supplying conduit artery. Although this is the most relevant physiological stimulus for flow-mediated dilation (FMD), the fluctuating pattern of shear that occurs may influence the shear stress-FMD stimulus response relationship. This study tested the hypothesis that the brachial artery FMD response to a step increase in shear is influenced by the fluctuating characteristics of the stimulus, as evoked by forearm exercise. In 16 healthy subjects, we examined FMD responses to step increases in shear rate in three conditions: stable shear upstream of heat-induced forearm vasodilation (FHStable); fluctuating shear upstream of heat-induced forearm vasodilation and rhythmic forearm cuff inflation/deflation (FHF Fluctuating); and fluctuating shear upstream of exercise-induced forearm vasodilation (FEStep Increase). The mean increase in shear rate (±SD) was the same in all trials (FH Fluctuating: 5.16 ± 5.70 s−1; FH Stable: 5.216 ± 14.10 s−1; FEStep Increase: 50.14 ± 13.03 s−1 P = 0.131). However, the FH Fluctuating and FE Step Increase trials resulted in a fluctuating shear stress stimulus with rhythmic high and low shear periods that were 96.18 ± 24.54 and 11.80 ± 7.30 s−1, respectively. The initial phase of FMD (phase I) was followed by a second, delayed-onset FMD and was not different between conditions (phase I: FH Fluctuating: 5.63 ± 2.15%; FH Stable: 5.33 ± 1.85%; FEStep Increase: 5.30 ± 2.03%; end-trial: FH Fluctuating: 7.76 ± 3.40%; FH Stable: 7.00 ± 3.03%; FEStep Increase: 6.68 ± 3.04%; P = 0.196). Phase I speed also did not differ (P = 0.685). In conclusion, the endothelium transduced the mean shear when exposed to shear fluctuations created by a typical handgrip protocol. Muscle activation did not alter the FMD response. Forearm exercise may provide a viable technique to investigate brachial artery FMD in humans.

Doppler ultrasound; mechanotransduction; forearm exercise; blood flow

A PROPERLY FUNCTIONING VASCULAR endothelium is essential for vasoregulation and vascular health. In healthy arteries, an increase in blood flow-associated shear stress results in the local release of endothelial factors, which cause an endothelium-dependent, flow-mediated dilation (FMD) (4, 23, 29). There are several shear stress elevation techniques, each with a distinct shear stress profile, that have been employed to investigate FMD in human conduit arteries. These include large transient increases in shear stress following the release of temporary limb occlusion (reactive hyperemia) (4, 5); more moderate, gradual, and sustained increases brought about by limb heating or distal acetylcholine infusion (1, 14, 19); and moderate step increases brought about by limb heating with the addition of controlled arterial compression (24). These shear stress profiles differ in their rate of onset, their peak magnitude, and their duration. Furthermore, these disparate stimuli can evoke distinct FMD mechanisms, dynamics, and magnitudes (1, 15, 19). Thus the FMD response characteristics appear to be sensitive to the nature of the shear stimulus profile (26).

Exercise is arguably the most common cause of substantive shear stress increase in conduit arteries in vivo. Exercise evokes a rapid reduction in vascular resistance in the muscle vascular bed, which results in sustained increases in upstream conduit artery blood flow that are exercise intensity dependent (7). Exercise could provide a convenient way to study FMD in response to prolonged increases in shear stress, without drug infusion or limb heating interventions. It has been used to create the shear stimulus in a small number of studies examining FMD (11, 21). However, the potential confound of the fluctuating pattern of exercise shear stress has not been considered. The fluctuating pattern is created by contraction-induced impedance to flow, which can include a brief period of reverse flow, and a relatively enhanced hyperemia during relaxation. Exposure to oscillatory flow (repetitive forward reverse cycles) has been shown to influence endothelial vasodilatory function in vitro and ex vivo (12, 38). However, it is currently unclear how acute exercise-induced fluctuations in shear stress impact the FMD response in human conduit arteries. Specifically, it is unclear whether the endothelium responds to the mean shear stress or whether it is disproportionately influenced by the high or low periods of the fluctuating shear stimulus profile.

In addition to the distinct characteristics of the exercise shear stress stimulus, exercise can increase sympathetic nervous system activity (3), and this might influence conduit vessel tone and potentially blunt the FMD response (8, 13). Exercise also changes the metabolic environment of the muscle, resulting in arteriolar vasodilation at the site of metabolic demand. Initial vasodilation originating in the terminal arterioles directly accessible to local metabolic vasodilator influences in activated skeletal muscle can also conduct up the arterial tree, resulting in dilation of more remote vessels (35). It is currently unknown if this conducted vasodilation can reach the level of conduit arteries in humans; however, it must be considered as a possible nonshear contributor to observed conduit vessel dilation in response to exercise.

With this as a background, the objectives of the present study were 1) to identify if there is a distinct FMD response to a stable (nonfluctuating) vs. a forearm exercise (FE)-induced
increase in brachial artery shear stress; and 2) to isolate the effects of the fluctuating characteristics of the shear stress pattern created with exercise from the other consequences of muscle activation. These manipulations provide insight to evaluate whether exercise-induced increases in shear stress can be used to assess endothelial function (via FMD). To achieve this, we created a step increase in brachial artery blood velocity to the same (within-subjects) mean target in three distinct conditions: 1) stable stimulus via forearm heating (FH); 2) fluctuating stimulus with muscle activation via FE; and 3) fluctuating stimulus with no muscle activation via FH and rhythmic mechanical forearm compression. A fourth condition included exercise with no increase in shear to isolate any possible conducted vasodilation. Our results suggest that 1) the brachial artery endothelium transduces the mean shear when exposed to shear fluctuations created by a typical handgrip protocol; and 2) muscle activation does not alter the FMD response. FE may, therefore, provide a viable technique to investigate brachial artery FMD in humans.

METHODS

Subjects

Sixteen healthy, nonsmoking male subjects, between the ages of 19 and 35 yr, from the Queen’s University community, 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 procedures were approved by the Health Sciences Human Research Ethics board at Queen’s University, which operates under the terms of Helsinki, 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 participated in testing over 3 days. The conditions in which blood flow was elevated passively were performed on day 1, and the exercise conditions were performed on day 2 and repeated on day 3. All experiments were performed at the same time of day (±2 h) in a quiet, temperature-controlled room (22°C heating; 18°C exercise). 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 16 days.

Subject monitoring. Heart rate was monitored throughout each study via three-lead ECG. Blood pressure was measured continuously via Finapres (Ohmeda 2300).

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). All scans were performed at an insonation angle of 68° for reasons discussed previously (27). 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 supported with a clamp stand and a guide adhered to the skin. The brachial artery was imaged by two-dimensional gray-scale ultrasound imaging in B-mode with the same probe operating at 10 MHz. The probe operator was able to make corrections to probe placement to maintain an optimal velocity signal and vessel image throughout the experiment. The images were recorded in Digital Imaging and Communications in Medicine format for future analysis with custom-automated, edge-detection software (36).

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.

FH. Up to the level of the antecubital fossa, the forearm was enclosed in a custom water bath. 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 pumped in to fill the bath. Once full, water was continually circulated between the water heater and the forearm water bath. 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. During the heating trials at the initiation of water bath filling, the subject’s brachial pulse was located just proximal to the antecubital 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. The compression acted as a dam, allowing control of upstream blood velocity. 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 a desired target. Due to subject movement during the exercise trials, when required, arterial compression was achieved via manual experimenter finger pressure applied to the brachial pulse.

Brachial artery shear stimulus conditions. FHFLUCTUATING. For FH-evoked fluctuating brachial artery shear stimulus (FHFLuctuating), to mimic the pattern of brachial artery blood velocity created with FE, an inflatable cuff was placed on the forearm just distal to the antecubital fossa during heating. Upon release of arterial compression, the cuff was rhythmically inflated and deflated (duty cycle: 2-s inflation to 3-s deflation). Pilot work indicated that the specific manner of inflation/deflation required to mimic FE involved immediate inflation to 150 mmHg and a rapid reduction to 100 mmHg, followed by rapid deflation for each inflation/deflation cycle.

FHSTABLE. For FH-evoked stable brachial artery shear stimulus (FHStable), step increases in brachial artery blood velocity were achieved via controlled release of the arterial compression stylus. Blood velocity was targeted to achieve the same mean blood velocity created in the FHFLuctuating condition.

FE-EVOKED BRACHIAL ARTERY SHEAR STIMULUS. Before the initiation of FE trials, subjects first performed three maximum voluntary contraction (MVC) attempts. Subjects then performed a series of contractions at different intensities to identify the contraction intensity (%MVC) that elicited the same mean brachial artery blood velocity achieved during the FHFLuctuating condition. During the FE trials, isometric handgrip force feedback was displayed continuously for the subjects on a computer data-acquisition system (Powerlab; ADInstruments). Subjects achieved the target force and duration for each contraction by displacing the force readout line to the desired level in time with a 2-s contraction/3-s relaxation duty cycle beeper. Brachial artery blood velocity was continually monitored throughout the trial, and experimenters coached the subjects through minor increases and decreases in force production to maintain the desired blood velocity target.

Experimental Protocols

There were a total of five different shear stimulus condition protocols, which all had in common the following (see Fig. 1). Baseline brachial artery images and blood velocity before the initia-
tion of the increased shear stimulus were recorded for 1 min (FH precompression baseline and FE baseline). Blood velocity was monitored continuously throughout the protocol, and a minimum of 10 min were allowed between trials to permit brachial artery diameter to return to baseline. Specific to the FH trials, once filling of the water bath was initiated, arterial compression commenced, and baseline blood velocity was maintained during the initial heating period. To ensure a maximal reduction in forearm vascular resistance, heating and arterial compression were performed for 30 min before the first trial was started. One minute of baseline blood velocity and blood vessel diameter was recorded before blood velocity increases were initiated in the FH trial (FH baseline). Arterial compression was resumed between each trial to return blood velocity to baseline.

Arterial compression release (controlled for FHStable or complete for FHFluctuating) Arterial compression resumed Arterial compression release (controlled for FHStable or complete for FHFluctuating)

Manual arterial compression

FE. Subjects experienced three different exercise shear stimulus conditions on day 2 and repeated them on day 3. The two trials from each condition were averaged together to provide one mean subject response for each condition. The specific exercise shear stimulus conditions were as follows.

FEStep Increase. For FE step increase in shear stimulus (FEStep Increase), the brachial pulse was located, and finger pressure was used to perform arterial compression to maintain blood velocity at baseline for the first approximately five contractions. On arterial compression release, a step increase in blood velocity was achieved. This method was employed to ensure the same rapid shear stimulus onset as was achieved in the FHFluctuating and FHStable conditions. Contractions were performed for 10 min.

FENormal Increase. The FE normal rate of increase in shear stimulus (FENormal Increase) condition was performed as a control to determine

Fig. 1. A: day 1 protocol timeline. B: day 2 and 3 protocol timeline. Subjects performed each of the two forearm heating (FH) trials twice on day 1. Subjects performed all three forearm exercise (FE) trials on day 2 and repeated them on day 3. See METHODS for definition of FHFluctuating, FHStable, FEStep Increase, FENormal Increase, and FENo Increase.
whether the FE\textsubscript{Step Increase} FMD response differed from the FMD response to a normal rate of increase in brachial artery shear stimulus at exercise onset. In this condition, no arterial compression was performed at the onset of contractions, and this resulted in the normal, more gradual increase in blood velocity with exercise. Contractions were performed for 10 min.

\textbf{FE\textsubscript{ISO Increase}.} The FE where shear stimulus increase is prevented (FE\textsubscript{ISO Increase}) condition was designed to assess whether mechanisms of vasodilation other than brachial artery shear-induced FMD were at work in the conduit artery. Handgrip exercise (intensity matched to FE\textsubscript{Step Increase} condition) was performed for 4.5 min during manual arterial compression (finger pressure), maintaining blood velocity at baseline.

\textbf{Data Analysis}

\textbf{Brachial artery blood velocity.} Blood velocity was analyzed offline in 3-s average time bins. A mean blood velocity profile for each subject in each condition was obtained from an average of the two trials per condition.

\textbf{Brachial artery diameter.} Vessel diameter was analyzed using an updated version of the automated edge-detection software package (FMD/blood flow acquisition and analysis), described in Woodman et al. (36). 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 program allows for the removal of erroneous data points due to vessel wall tracking errors.

The diameter data were compiled as 3-s time bins, allowing multiple trials to be time aligned and averaged. Missing data due to tracking error in individual trials were interpolated to facilitate calculation of the average. These averaged data were then plotted over time, and a line of best fit was applied using custom software that allows the user to select the type of exponential function and manipulate the function parameters to form a curve that is the best fit for the data. The function parameters are 1) the number of phases, 2) the time delay (TD), which is the time between the onset of the shear stimulus and the onset of the response for that phase, and 3) the time constant (τ), an index of response speed, which is the time to 63% of the maximal response (Fig. 2). Using the 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) or absolute change in diameter [FMD\textsubscript{absolute} (mm)] from the baseline measurement before release of arterial compression (FH\textsubscript{Fluctuating} and FH\textsubscript{Stable}) or before commencement of exercise (FE\textsubscript{Step Increase}, FE\textsubscript{Normal Increase}-FE\textsubscript{ISO Increase}).

\textbf{Shear rate.} Shear rate (an estimate of shear stress without viscosity) was calculated as mean blood flow velocity/vessel diameter and was used to quantify the shear stimulus for FMD. The 3-s average shear rate was averaged 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 10-min shear stimulus period in each condition.

\textbf{Statistical analysis.} Repeated-measures ANOVA and paired \( t \)-tests were used to compare the shear rate and FMD response (%change in diameter, delta, TD, and response speed) parameters. To test the hypothesis that the brachial arterial wall would respond to the mean shear stimulus, the FH\textsubscript{Fluctuating}, FH\textsubscript{Stable}, and FE\textsubscript{Step Increase} conditions were compared. FE\textsubscript{Step Increase} and FE\textsubscript{Normal Increase} were compared separately to evaluate the impact of the FE\textsubscript{Normal Increase} slower shear stimulus onset. To test the hypothesis that increases in brachial artery diameter during FE were due to FMD and not other vasodilatory mechanisms, within the FE\textsubscript{ISO Increase} condition, parameters were compared between baseline and during exercise. The level for significance was set at \( P < 0.05 \), and significant differences for repeated-measures ANOVA were further assessed using Tukey’s post hoc tests. All statistics were calculated using SigmaStat 2.03 (SPSS, Chicago, IL), with the exception of regression slope and intercept comparisons, which were performed in GraphPad Prism 5. All data are expressed as means ± SD.

\textbf{RESULTS}

\textbf{Heart Rate}

Baseline heart rate was not significantly different between the two FH conditions (FH\textsubscript{Fluctuating} vs. FH\textsubscript{Stable}; \( P = 0.577 \)) (Table 1). However, baseline heart rate was significantly lower in the exercise condition vs. the passive conditions (\( P < 0.001 \)). Heart rate only changed significantly from baseline during exercise (\( P < 0.001 \)).

\textbf{Blood Pressure}

Baseline mean arterial pressure (MAP) was not significantly different between trials (Table 1). MAP changed modestly during passive elevations in blood velocity [decreased \( \sim 3 \) mmHg in FH\textsubscript{Fluctuating} (\( P = 0.011 \)) and increased \( \sim 2 \) mmHg in FH\textsubscript{Stable} (\( P = 0.108 \))], while handgrip exercise (29.19 ± 6.29% MVC) resulted in a significant \( \sim 12\)-mmHg elevation in MAP (\( P < 0.001 \) vs. baseline).

\textbf{Brachial Artery Shear Rate Pattern: FH\textsubscript{Fluctuating} vs. FE\textsubscript{Step Increase}}

A sample raw velocity trace is shown in Fig. 3. As expected with a human in vivo investigation, all conditions exhibit a cardiac cycle-induced pulsatile blood velocity profile. Superimposed on this normal in vivo pulsatile velocity, the exercise condition (FE\textsubscript{Step Increase}) has periods of high (3-s relaxation) and low (2-s contraction) velocity. The low-velocity period also includes a brief period (approximately less than one-half a cardiac cycle) of reverse flow (indicated by the arrows in Fig. 3). This is a typical fluctuating exercising blood velocity pattern. To confirm that the pattern of the shear stimulus in the
Flow, the average shear rate during the reverse period was quantified in a subset of 10 subjects. The average shear rate of the reverse period was not significantly different between conditions (FHfluctuating: −45.90 ± 13.65 s⁻¹; FEStep Increase: −43.10 ± 8.26 s⁻¹; P = 0.592). This indicates that the overall reverse shear stimulus was similar in the two conditions.

### Brachial Artery Shear Rate

The brachial artery shear rate profile is displayed in Fig. 4. Baseline shear rate was not significantly different between the two FH conditions (FHfluctuating: 12.68 ± 3.26 s⁻¹ vs. FHStable: 13.04 ± 4.21 s⁻¹; P = 0.953). FEStep Increase baseline shear rate (16.01 ± 5.82 s⁻¹) was significantly greater than FHfluctuating (P = 0.028), but did not achieve statistical significance vs. FHStable (P = 0.053). Average shear rate during the 10-min shear stimulus period was slightly but significantly higher in FEStep Increase (66.15 ± 16.82 s⁻¹) vs. FHfluctuating (64.37 ± 15.55 s⁻¹) (P = 0.018). Average shear rate during the 10-min shear stimulus period in FHStable (65.21 ± 15.32 s⁻¹) was not significantly different from either FHfluctuating or FEStep Increase (P = 0.369 and 0.284, respectively). Given that the baseline shear rate was different between conditions, we also calculated the change in shear rate from baseline. The change in shear rate was not significantly different between trials (FHfluctuating: 51.69 ± 15.70 s⁻¹; FHStable: 52.16 ± 14.10 s⁻¹; FEStep Increase: 50.14 ± 13.03 s⁻¹; P = 0.131).

Due to potential early differences in the shear stimulus magnitude, the area under the curve (AUC) of the shear rate in first 60 s of the shear stimulus period (60-s shear rate AUC) was calculated for each trial. There was no significant difference between conditions (FHfluctuating: 4.058,45 ± 1,162,22;

### Table 1. Heart Rate and Mean Arterial Pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End Trial</td>
</tr>
<tr>
<td>FHfluctuating</td>
<td>61.85 ± 7.08</td>
<td>61.74 ± 7.61</td>
</tr>
<tr>
<td>FHStable</td>
<td>61.91 ± 6.67</td>
<td>62.12 ± 6.67</td>
</tr>
<tr>
<td>FEStep Increase</td>
<td>57.76 ± 7.85†</td>
<td>63.81 ± 7.16*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. End-trial values are the average of the last 2 min of the stimulus period in each condition. FHfluctuating, forearm heating-evoked fluctuating brachial artery shear stimulus; FHStable, forearm heating-evoked stable brachial artery shear stimulus; FEStep Increase, forearm exercise step increase in shear stimulus. *Significantly different from baseline, P < 0.001. †Significantly different from FHfluctuating and FHStable, P < 0.001.

**Fig. 3.** Representative velocity trace from one subject. All three trials illustrate in vivo pulsatility. The FHfluctuating condition and FEStep Increase condition have fluctuating 3-s periods of "high" shear (above the mean) during cuff release/relaxation, alternating with 2-s periods of "low" shear (below the mean) during cuff inflation/contraction. The arrows indicate the burst of reverse shear that occurs due to mechanical compression of the forearm by cuff inflation or muscle contraction. The FHStable condition has no fluctuations and is controlled at the target mean shear with arterial compression.
Baseline Brachial Artery Diameter

Baseline diameter was not significantly different between the two FH conditions (FH Fluctuating: 3.83 ± 0.51 mm vs. FH Stable: 3.84 ± 0.51 mm; P = 0.862). In addition, baseline diameter before heating and arterial compression (precompression baseline: 3.82 ± 0.49 mm) was not significantly different than baseline diameter in either of the heating trials (P = 0.584). However, FE Step Increase baseline diameter (3.75 ± 0.49 mm) was significantly smaller than that in either of the two FH conditions (P < 0.001).

FMD

Response dynamics. The average response profile is shown in Fig. 5. Only the phase I FMD dynamics are reported as variability in the diameter adaptation in the late portion of the trial, which made fitting a clear exponential adaptation unreliable. The TD between the onset of the shear stimulus and the onset of the FMD response in FH Fluctuating was significantly shorter than the other conditions (Fig. 6, top). Furthermore, regardless of the pattern (fluctuating vs. stable) or mode (passive vs. exercise) of shear stimulus increase, the TD did not depend on the stimulus magnitude, demonstrated by the lack of relationship between these variables (TD vs. shear magnitude: FH Fluctuating r² = 0.05, P = 0.402; FH Stable r² = 0.21, P = 0.073; FE Step Increase r² = 0.168, P = 0.114). The speed of the FMD response (τ) was unaffected by the mode of shear stimulus increase (P = 0.685) (Fig. 6, bottom). Furthermore, similar to the TD, the speed of the response did not depend on the magnitude of the stimulus (τ vs. shear magnitude: FH Fluctuating r² = 0.12, P = 0.187; FH Stable r² = 0.075, P = 0.304; FE Step Increase r² = 0.081, P = 0.285).

Response magnitude. There was no difference in the response magnitude between conditions (%FMD P = 0.196 and FMD absolute P = 0.111), although the end-trial FMD was significantly larger than the phase I FMD magnitude (P = 0.001) (Fig. 7, top left and top right). Furthermore, the relationship between the stimulus magnitude and the response magnitude (phase I and end trial) was not different between the different conditions (phase I: FH Fluctuating r² = 0.53, P = 0.001; FH Stable r² = 0.61, P < 0.001; FE Step Increase r² = 0.36, P = 0.013; no slope difference P = 0.975, no Y-intercept difference P = 0.793; end trial: FH Fluctuating r² = 0.57, P < 0.001; FH Stable r² = 0.30, P = 0.03; FE Step Increase r² = 0.34, P = 0.018; no slope difference P = 0.747, no Y-intercept difference P = 0.539; Fig. 7, bottom left and bottom right). The phase I response accounted for 76.26 ± 19.13, 80.63 ± 20.26, and 84.20 ± 19.90% of the end-trial response magnitude in the FH Fluctuating, FH Stable, and FE Step Increase conditions, respectively (P = 0.375).
FEStep Increase vs. FENormal Increase. The FEStep Increase and FENormal Increase parameter averages are reported in Table 2. The only significant differences were in the 60-s shear rate AUC and the TD of the phase I vasodilatory response. This indicates that the FENormal Increase condition had a smaller initial shear stimulus magnitude (as expected due to the slower onset) (Fig. 8), which resulted in a delayed onset of FMD, but had no impact on response speed or magnitude.

Table 2. FEStep Increase and FENormal Increase parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FEStep Increase</th>
<th>FENormal Increase</th>
<th>( P ) Value</th>
</tr>
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<tbody>
<tr>
<td>Baseline HR, beats/min</td>
<td>57.76 ± 8.71</td>
<td>58.57 ± 8.71</td>
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<td>End-trial HR, beats/min</td>
<td>63.81 ± 7.16</td>
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<td>Baseline MAP, mmHg</td>
<td>84.59 ± 8.57</td>
<td>87.31 ± 4.98</td>
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<tr>
<td>End-trial MAP, mmHg</td>
<td>96.33 ± 10.98</td>
<td>97.59 ± 8.97</td>
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<tr>
<td>Baseline shear rate, s(^{-1})</td>
<td>16.01 ± 5.82</td>
<td>17.58 ± 5.33</td>
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<tr>
<td>Average exercise shear rate, s(^{-1})</td>
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<td>65.67 ± 15.88</td>
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<tr>
<td>60-s Shear rate AUC</td>
<td>4,108.74 ± 1,131.19</td>
<td>3,583.95 ± 882.45</td>
<td>&lt;0.001*</td>
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<td>Baseline diameter, mm</td>
<td>3.75 ± 0.49</td>
<td>3.75 ± 0.50</td>
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<td>Time delay, s</td>
<td>34.93 ± 9.09</td>
<td>46.30 ± 17.78</td>
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<td>( \tau ), s</td>
<td>25.29 ± 23.29</td>
<td>27.01 ± 17.87</td>
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<tr>
<td>Phase I %FMD</td>
<td>5.30 ± 2.03</td>
<td>5.19 ± 1.74</td>
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<td>Phase I FMDrelative, mm</td>
<td>0.20 ± 0.06</td>
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<tr>
<td>End-trial %FMD</td>
<td>6.68 ± 3.04</td>
<td>6.44 ± 2.97</td>
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<td>Force, %MVC</td>
<td>29.19 ± 6.29</td>
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<td>Relaxation shear rate, s(^{-1})</td>
<td>96.36 ± 24.90</td>
<td>95.67 ± 24.87</td>
<td>0.360</td>
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<tr>
<td>Contraction shear rate, s(^{-1})</td>
<td>12.55 ± 7.96</td>
<td>12.43 ± 7.35</td>
<td>0.853</td>
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<tr>
<td>Nadir in shear rate, s(^{-1})</td>
<td>−74.90 ± 16.00</td>
<td>−75.64 ± 18.35</td>
<td>0.777</td>
</tr>
</tbody>
</table>

Values are means ± SD. FENormal Increase, forearm exercise normal rate of increase in shear stimulus; HR, heart rate; MAP, mean arterial pressure; AUC, area under the curve; \( \tau \), time constant; FMD, flow-mediated dilation; MVC, maximal voluntary contraction. *Significant difference.

Fig. 7. FMD response magnitude. Top left: relationship between the increase in shear rate from baseline and the phase I %FMD: \( r^2 = 0.49, P < 0.001 \). Bottom left: relationship between the increase in shear rate from baseline and the phase I %FMD with individual regressions for each condition. See text for individual \( r^2 \) values. Top right: relationship between the increase in shear rate from baseline and the end-trial %FMD: \( r^2 = 0.40, P < 0.001 \). Bottom right: relationship between the increase in shear rate from baseline and the end-trial %FMD with individual regressions for each condition. See text for individual \( r^2 \) values. Small symbols indicate individual subject data, and large symbols indicate group mean data, as indicated in the graph legend. Bidirectional error bars represent SD.

FENo Increase. See Fig. 9. Baseline heart rate in the FE No Increase condition was 57.22 ± 8.28 beats/min. Heart rate rose significantly from baseline and was 67.06 ± 7.88 beats/min in the last 30 s of exercise (\( P < 0.001 \) vs. baseline). Baseline blood pressure in the FE No Increase condition was 92.89 ± 8.08 mmHg. Blood pressure rose over the exercise period and was 116.93 ± 15.58 mmHg in the last 30 s of exercise (\( P < 0.001 \) vs. baseline). Despite the application of...
Fig. 8. Stimulus initiation in the FEStep Increase vs. FENormal Increase conditions. Arterial compression resulted in a fast stimulus onset in the FEStep Increase condition, while the stimulus onset followed a slower profile in the FENormal Increase condition, where blood flow increased normally in response to exercise. Each data point represents a 21-s average. Values are means ± SD. *The 60-s shear rate AUC is significantly different between conditions.

arterial compression, shear rate was slightly higher during exercise vs. baseline (19.54 ± 4.34 vs. 14.89 ± 3.00 s⁻¹, P < 0.001). Brachial artery diameter did not change from baseline during the exercise period [3.74 ± 0.52 vs. 3.71 ± 0.54 mm (±0.80 ± 1.75%)] (P = 0.094).

**DISCUSSION**

This study was designed to investigate the FMD response to stable FH-induced vs. fluctuating forearm handgrip exercise-induced increases in brachial artery shear stimulus. Specifically, it isolated the effect of the shear stress pattern created by exercise from factors associated with muscle activation. The primary findings were that 1) the FMD response magnitude, the stimulus-response relationship, and the response speed were determined by the mean shear, independent of the shear stimulus pattern (fluctuating vs. stable) and presence of muscle activation (heating vs. exercise); and 2) conducted vasodilation did not contribute to the brachial artery response to handgrip exercise. These data indicate that with a typical handgrip exercise shear stimulus pattern, 1) the endothelium transduced the mean shear stimulus; and 2) the FMD response was unaffected by muscle activation. Although future research must be directed at investigating FMD in response to a range of duty cycles and exercise intensities, our results suggest that exercise may provide a viable means to increase shear to investigate FMD in humans.

**Impact of a Fluctuating Shear Stress Pattern on FMD**

Our group (25) and others have previously shown that sustained increases in shear stress, created with distal heating (1, 14, 19) or exercise (31, 37), result in conduit artery (brachial or radial) FMD that is positively related to the magnitude of the shear stimulus. However, to our knowledge, no other study has matched the pattern of shear stress in a FH vs. exercise condition. In the present experiment, the fluctuating conditions (FH_fluctuating and FEStep Increase) consisted of a shear stimulus during relaxation/cuff deflation that was higher, and a shear stimulus during contraction/cuff inflation that was lower, than the mean shear (Fig. 3). Thus there were marked, rhythmic fluctuations in shear compared with the mean value (mean ~65 s⁻¹; high phase ~96 s⁻¹; low phase ~12 s⁻¹). In addition, the “low phase” was also characterized by a brief period (approximately less than one-half a cardiac cycle) of reverse flow. If the endothelium was responding to the “high phase”, we would have expected to see greater FMD in the fluctuating conditions (FH_fluctuating and FEStep Increase) vs. the stable condition (FH_Stable). Conversely, if the endothelium was responding to the “low phase” or the reverse flow, we would have expected to see attenuated FMD in the fluctuating conditions vs. the stable condition. Again, the observation that the FMD response was the same in all conditions indicates that, with a 2-s contraction/3-s relaxation duty cycle, the endothelium responds to the mean level of shear stimulus and is unaffected by transient fluctuations around that mean. Future investigations are required to determine whether this finding is consistent across different exercise intensities and frequencies of fluctuation created by different duty cycles.

Although no previous studies have specifically investigated the impact of the shear stimulus pattern on human FMD, this phenomenon has been investigated in a small number of animal studies (16, 33, 34). In agreement with the present study, it has been shown in vivo (16) and in isolated vessels (34) that the magnitude of FMD evoked by an elevation in mean shear with small pulsatile amplitude is not altered when pulsatile amplitude is increased. However, in contrast with our human in vivo observations (Fig. 7), Kelly and Snow (16) report a blunted FMD response to the initiation of an increase in mean shear with a high- vs. a low-pulsatile amplitude. This effect may be species or frequency dependent.

The impact of shear direction on FMD has also been investigated previously. In isolated vessels, exposure to reversing oscillatory shear (no net forward shear) did not result in FMD (34), and prolonged exposure to this type of stimulus impaired subsequent endothelial-dependent nitric oxide (NO)-mediated vasodilation (12). Additionally, FMD is reduced in response to maintained shear in the reverse vs. forward direction (17, 18). Application of superoxide dismutase (34) or an superoxide dismutase mimetic (tempol) (17) has been shown to normalize responses to that of the forward shear condition. Therefore, the negative effects of reverse shear may result from reduced NO bioavailability caused by superoxide anion production.

Fig. 9. Mean arterial pressure (MAP), shear rate, and change in brachial artery diameter during the exercise period of the FEStep Increase Condition. Each data point represents a 3-s average. Values are means ± SD.
These reverse shear findings underscored the need to evaluate the effect of an exercise shear pattern on FMD. Our observation that the response was the same with fluctuating vs. stable stimulus patterns indicates that the minor shear reversals with the reported handgrip protocol do not impair FMD. Furthermore, if free radicals are produced with the reverse shear, there is evidence that, in human handgrip, they contribute to rather than hinder the dilatory response in the brachial artery (28).

**FMD Response Dynamics**

The dynamics of the response were only characterized for the first phase of diameter adaptation (phase I FMD). The speed ($\tau$) of this first phase of dilation was the same in all conditions, and this suggests that the mechanisms of the response were the same, regardless of the pattern of the shear stimulus (fluctuating vs. stable) or presence of muscle activation (heating vs. exercise). In contrast, the TD between the onset of the shear stimulus and the initiation of the FMD response was significantly different between the FH\textsubscript{Fluctuating} vs. FH\textsubscript{Stable} and FE\textsubscript{Step Increase} conditions (Fig. 6, top). This could be because the FH\textsubscript{Fluctuating} condition was characterized by a larger percent overshoot in shear rate (defined as the percent difference between the first 21-s time bin and the average shear rate). However, this is unlikely because these variables (overshoot vs. TD) were only very weakly negatively correlated ($r^2 = 0.131$).

Taken together, the data suggest that, while the time that it takes to initiate the mechanisms responsible for the FMD may depend on some interaction between the mode and pattern of shear stimulus increase, the consistent speed of the first phase across conditions suggests similar mechanisms are involved. However, it must be acknowledged that this cannot be confirmed with the noninvasive design of the present study. Further mechanistic studies are required to elucidate the roles of NO, endothelial derived hyperpolarizing factor (1, 19), and free radicals (10, 22, 28) in FMD associated with passive vs. exercise-induced increases in shear stress.

**Step vs. Normal Rate of Increase in Exercise-Induced Brachial Artery Shear**

This study was designed to match the shear stimulus pattern created by exercise with a passive shear stress elevation technique (FH\textsubscript{Fluctuating} condition). In normal exercise, blood flow does not increase in a step fashion at the onset of contractions. However, in this study, we compared step increases in the shear stimulus, which facilitates the quantification of the response onset (TD) and rate of response adaptation ($\tau$). We compared the FE\textsubscript{Step Increase} condition to the normal, slower increase in blood flow that occurs with exercise (FE\textsubscript{Normal Increase}) to determine whether that slow shear stimulus onset has a role in the response. We found that there was a clear difference in the rate of shear stimulus onset [60-s shear rate AUC (Table 2) and Fig. 8] and a resultant slower “turning on” of the mechanisms responsible for FMD (TD only). Future research is required to clarify the mechanisms responsible for this response, but our results suggest that, once FMD mechanisms are initiated, they are relatively insensitive to small differences in the rate of shear stimulus increase.

**Potential Non-FMD Contributions to Changes in Brachial Artery Tone**

Vasodilation of resistance vessels upstream from the site of elevated muscle metabolism with contractions can be achieved by rapid cell-to-cell conduction of vasodilation (9, 20, 30). In the hamster cremaster muscle, Berg et al. (2) observed a rapid gap junction-dependent dilation of upstream arterioles that persisted at least three branch points removed from a capillary bed in contact with stimulated muscle. Previous studies that have reported brachial artery vasodilation in response to handgrip exercise acknowledge that it is possible that some of the observed vasodilation may be the result of this type of signal conducted up the vascular tree (28, 31, 37). However, it is presently unknown if conducted vasodilation can reach the level of conduit arteries. Therefore, it was necessary to identify any possible contribution of conducted vasodilation in the exercise conditions.

In the FE\textsubscript{No Increase} condition, exercise was performed, creating the metabolic stimulus for conducted vasodilation, while brachial artery shear rate was controlled near baseline to eliminate FMD (Fig. 9). The observation that there was no vasodilation during the FE\textsubscript{No Increase} condition suggests that the vasodilation observed in the FE\textsubscript{Step Increase} or FE\textsubscript{Normal Increase} conditions is, in fact, flow mediated and not due to a conducted vasodilation.

The increase in MAP (~24 mmHg) in the FE\textsubscript{No Increase} condition could indicate a confounding increase in sympathetic activation of the brachial artery (Fig. 9). However, Shoemaker et al. (32) recently investigated the mechanisms responsible for increases in blood pressure in response to ischemic handgrip exercise performed in the supine position. Their results indicated that the observed increase in MAP (~20 mmHg) was due to cardiac output mediated by an increase in both heart rate and stroke volume and not an increase in peripheral vasoconstriction. In the FE\textsubscript{No Increase} condition, heart rate increased by ~17% and MAP increased by ~25%. This supports the position that our observed increases in blood pressure can largely be explained by cardiac output, thus minimizing the concern that sympathetically mediated vasoconstriction may have impaired detection of a conducted vasodilation. Furthermore, examination of the time course of the FMD indicates that phase I was virtually fully expressed in the normal exercise condition before there was any substantive increase in MAP in the FE\textsubscript{No Increase} condition. Thus we believe that the FE\textsubscript{No Increase} model provides a valid confirmation that conducted vasodilation does not contribute to the exercise response in the brachial artery.

**Potential Clinical Significance of FMD in Response to Exercise**

The majority of previous work demonstrating that FMD is impaired in at-risk/diseased populations has utilized the reactive hyperemia methodology (4, 6). However, exercise represents a more physiological stimulus for FMD, and the findings of the present study indicate that muscle activation does not confound the results. Currently, however, little is known about the relationship between exercise-induced FMD and vascular health. Padilla et al. (21) found that, following the ingestion of a high fat meal (a known perturbation to endothelial function), FMD in response to reactive hyperemia was blunted, while
FMD in response to handgrip exercise was unchanged. However, Gazner et al. (11) found that both femoral artery FMD in response to cycling exercise and the brachial artery FMD response to reactive hyperemia were impaired in smokers. Further study is required to clarify whether exercise tests can provide distinct clinically relevant information regarding endothelial function. Pending the results of these future investigations, exercise may become a valuable new tool with which to evaluate endothelial function.

Conclusions

We found that, when the brachial artery endothelium was exposed to the same mean shear stimulus, the magnitude and speed of the FMD response were unaffected by either the shear stimulus pattern (fluctuating vs. stable) or presence of muscle activation (heating vs. rhythmic handgrip exercise). We conclude that, during a typical handgrip exercise protocol, the human brachial artery endothelium transduced the mean shear stimulus and that the response was sheared mediated with no contributions from conducted vasodilation.

Exercise is a physiologically relevant and methodologically simple means to create a sustained shear stimulus, and our results suggest that it may provide a viable option for creating a stimulus in human FMD investigations. Future research is required to investigate 1) the impact of a range of duty cycles and exercise intensities; and 2) the connection of exercise-induced FMD to vascular health.

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