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ORIGINAL ARTICLE

# Ramp and step increases in shear stress result in a similar magnitude of brachial artery flow-mediated dilation

**Joshua C. Tremblay**[1] · **Jennifer S. Williams**[1] · **Kyra E. Pyke**[1]

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## Abstract

**Purpose** There is evidence that the endothelium is responsive to both the rate and magnitude of increases in shear stress. However, whether flow-mediated dilation stimulated by sustained increases in shear stress (SS-FMD) is rate sensitive in humans is unknown. The purpose of this investigation was to test whether ramp (gradual) and step (instantaneous) increases in shear stress elicit disparate SS-FMD.

**Methods** Young, healthy men ($n = 18$, age = 22 ± 2 years, body mass index = 25 ± 3 kg m$^{-2}$) performed two 11-min bouts of rhythmic handgrip exercise; one with a 5.5-min ramp-increase in shear stress and one with an immediate step increase in shear stress. Ramp increases in shear stress were achieved through incremental increases in handgrip exercise intensity [increases of 4% maximum voluntary contraction (MVC) every 30 s for 5.5 min, ending at 44% MVC] and step increases in shear stress were achieved through a combination of arterial compression and commencing handgrip exercise at 44% MVC.

**Results** Shear rate was greater in the step versus ramp protocol in minutes 1–6, but not different thereafter. Similarly, SS-FMD was greater in the step versus ramp protocol during minutes 2–6, but similar in minutes 7–11 (minute 11: ramp 8.7 ± 4.6%; step 9.4 ± 3.6%; $P = 0.343$). SS-FMD continued to increase over time with maintenance of a steady shear stress stimulus (step minutes 2–11: 0.51 ± 0.36% min$^{-1}$; ramp minutes 7–11: 0.64 ± 0.57% min$^{-1}$; $P = 0.259$).

**Conclusions** These findings indicate that in the brachial artery of humans, the magnitude of SS-FMD is determined by the magnitude and duration, but not the rate, of increases in shear stress.

**Keywords** SS-FMD · Endothelial function · Handgrip exercise · Rate of increase · Rate sensitivity · Conduit artery

## Abbreviations

| | |
|---|---|
| FMD | Flow-mediated dilation |
| HR | Heart rate |
| MAP | Mean arterial pressure |
| MVC | Maximum voluntary isometric contraction |
| SS-FMD | Sustained stimulus flow-mediated dilation |

## Introduction

The endothelium plays an essential role in the regulation of arterial diameter. Both the magnitude and rate of increase in shear stress are sensed by a myriad of mechanotransducers within endothelial cells, initiating a cascade of signaling pathways leading to vasodilator production (Johnson et al. 2011; Butler et al. 2000; Frangos et al. 1996). In humans, measuring conduit artery dilation following exposure to sustained increases in shear stress created via limb heating or exercise [sustained stimulus flow-mediated dilation (SS-FMD)] is a versatile, physiologically relevant technique that provides distinct information regarding endothelial function compared to FMD stimulated by reactive hyperemia (Tremblay and Pyke 2018). However, the impact of the temporal gradient (i.e., the rate of increase) of shear stress on SS-FMD has not been investigated in humans.

Evidence from cell culture and isolated arteriole studies demonstrates a biphasic response of vasodilator production and subsequent FMD wherein the first phase is contingent

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✉ Kyra E. Pyke
pykek@queensu.ca

[1] Cardiovascular Stress Response Laboratory, School of Kinesiology and Health Studies, Queen's University, 28 Division Street, Kingston, ON K7L 3N6, Canada

on the rate of increase in shear stress, and the second is dependent on the magnitude of the steady-state shear stress (Frangos et al. 1996; Butler et al. 2000; Kuchan and Frangos 1994). In these studies, step (i.e., instantaneous) increases in shear stress elicit greater nitric oxide production (Frangos et al. 1996) and SS-FMD (Butler et al. 2000) compared to ramp (i.e., slower, gradual) increases to the same steady-state shear stress. In humans, Pyke et al. (2008b) observed similar handgrip exercise stimulated brachial artery SS-FMD in a step versus a slower, normal rise to steady-state shear stress (~60 s until steady-state), although these conditions resulted in relatively small differences in the rate of increase in shear stress. Whether a more pronounced difference in rate of increase in shear stress to the same steady-state influences brachial artery SS-FMD is unclear. This is an important gap in knowledge because identifying the influence of shear stress stimulus characteristics on endothelial responses is fundamental to understanding endothelial physiology and is also critical for informed design and interpretation of SS-FMD investigations in humans.

The purpose of the investigation was to determine the impact of the rate of increase in shear stress on brachial artery SS-FMD. Based on cell culture and animal findings, we hypothesized that a step-increase in shear stress would provoke greater SS-FMD compared to a slow, ramped-increase to a similar steady-state shear stress magnitude.

## Materials and methods

### Ethical approval

All experimental procedures and measurements were approved by the Queen's University Health Sciences Research Ethics Board, which conforms to the standards set by the Declaration of Helsinki (with the exception that this study was not registered in a database). Written informed consent was obtained on forms approved by this board prior to study participation.

### Participants and screening

Young, healthy men ($n = 18$, age = $22 \pm 2$ years, body mass index = $25 \pm 3$ kg $m^{-2}$) participated in the investigation. Given the mean SD (1.84%) of previous studies on SS-FMD from our laboratory (McPhee and Pyke 2018; Tremblay et al. 2018; King et al. 2013), the sample size required to detect a 1.5% difference in SS-FMD between step and ramp conditions with 80% power and alpha of 0.05 was estimated as 14. Participants were nonsmokers without cardiovascular disease and not taking any medications (determined via self-report on medical screening questionnaires). Prior to commencing the study, participants attended a screening visit. During the screening visit, participants were familiarized to the handgrip exercise ramp protocol, described below.

### Experimental design

Each participant attended one experimental visit, which included counterbalanced assessment of SS-FMD stimulated by (1) a step-increase in shear rate and (2) a ramp-increase in shear rate. The order of protocols alternated between participants as they were recruited (i.e. half of the initially recruited participants performed the step-increase protocol first and the other half performed the ramp-increase protocol first). Four participants were excluded from the final analysis due to inability to achieve 44% maximum voluntary isometric contractions (MVC) ($n = 2$), excessive participant movement ($n = 1$), or a weak blood velocity signal ($n = 1$). Of the 18 participants included in the final analysis, 10 completed the step-increase protocol first and eight completed the ramp-increase protocol first. Participants were fasted for a minimum of 6 h, abstained from caffeine and alcohol for a minimum of 12 h, and refrained from exercise for a minimum of 24 h before each testing session. Upon arrival, participants laid supine with their arms extended for a 30-min rest period. During the rest period, participants performed two MVC using a grip force transducer (MLT004/ST, ADInstruments, USA), with the higher of the two values taken as maximal. At the end of the rest period, participants took part in both the step and ramp SS-FMD conditions, separated by a minimum of 10 min or until diameter returned to baseline (Pyke and Jazuli 2011). Previous reports have demonstrated that SS-FMD (Pyke and Jazuli 2011) and FMD stimulated by reactive hyperemia (Barton et al. 2011; Pyke and Jazuli 2011) are repeatable within this time course. Specifically, Pyke and Jazuli (2011) found that SS-FMD did not change across four trials separated by approximately 10 min (trial 1 $7.3 \pm 3.6\%$, trial 2 $7.0 \pm 3.6\%$, trial 3 $6.5 \pm 3.5\%$, and trial 4 $6.8 \pm 2.9\%$, $P = 0.913$) and the intraclass correlation between these trials was 0.716.

### Experimental measurements

#### Heart rate and mean arterial pressure

Heart rate (HR) and mean arterial pressure (MAP) were continuously monitored throughout the vascular testing visit using a three-lead electrocardiogram and finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Netherlands), respectively. Both signals were recorded continuously in the program LabChart (ADInstruments, USA).

### Brachial artery diameter

Brachial artery diameter was obtained using two-dimensional ultrasound in B-mode (12 MHz, Vivid i2, GE Medical Systems, Canada). Ultrasound images were recorded with a VGA-to-USB frame grabber (Epiphan Systems Inc., Canada) and saved as audio video interleave files on a separate computer using commercially available software (Camtasia Studio, TechSmith, USA).

### Brachial artery blood velocity

Brachial artery blood velocity was obtained at an insonation angle of 68° using Doppler ultrasound operating at 4 MHz (Vivid i2, GE Medical Systems) as described previously (Pyke et al. 2008a). The Doppler shift frequency spectrum was analysed using a Multigon 500P TCD spectral analyser (Multigon Industries, USA) to determine the mean blood velocity. The resulting voltage output from the Multigon was continuously recorded (LabChart, ADInstruments) for subsequent analysis as previously described (Jazuli and Pyke 2011).

### Step SS-FMD (Fig. 1a)

Following 1-min of baseline brachial artery diameter and blood velocity recording, participants commenced handgrip exercise at 44% MVC at a duty cycle of 1 s contraction to 3 s relaxation. Cadence was provided via metronome accompanied by real-time visual feedback of grip force. To facilitate a step-increase in shear rate, arterial compression of the brachial artery distal to the site of ultrasound image acquisition was performed for the first four contractions (King et al. 2013; Pyke et al. 2008b). Participants continued rhythmic handgrip exercise at 44% MVC for a total of 11 min.

### Ramp SS-FMD (Fig. 1a)

Following 1-min of baseline brachial artery diameter and blood velocity recording, participants commenced handgrip exercise at 4% MVC at the same duty cycle as the step SS-FMD protocol (1 s contraction to 3 s relaxation). The % MVC increased incrementally by 4% every 30 s for 5.5 min (i.e. 0–30 s, 4%; 30–60 s, 8%; 60–90 s, 12%; 90–120 s, 16%; 120–150 s, 20%; 150–180 s, 24%; 180–210 s, 28%; 210–240 s, 32%; 240–270 s, 36%; 270–300 s, 40%; 300–330 s, 44%). Subsequently, rhythmic handgrip exercise was performed at 44% MVC for the remaining 5.5 min (11 min total).

## Data analysis

### Maximum voluntary contraction

The % MVC is reported for each 30-s interval of handgrip exercise during the step and ramp protocols.

### HR and MAP

HR is reported as the 1-min average recorded during the baseline minute of each SS-FMD test, and during each minute of handgrip exercise (minutes 1–11). MAP ($n = 16$; acquisition failure in $n = 2$) is presented as the change from the 1-min baseline period during each minute of handgrip exercise (minutes 1–11).

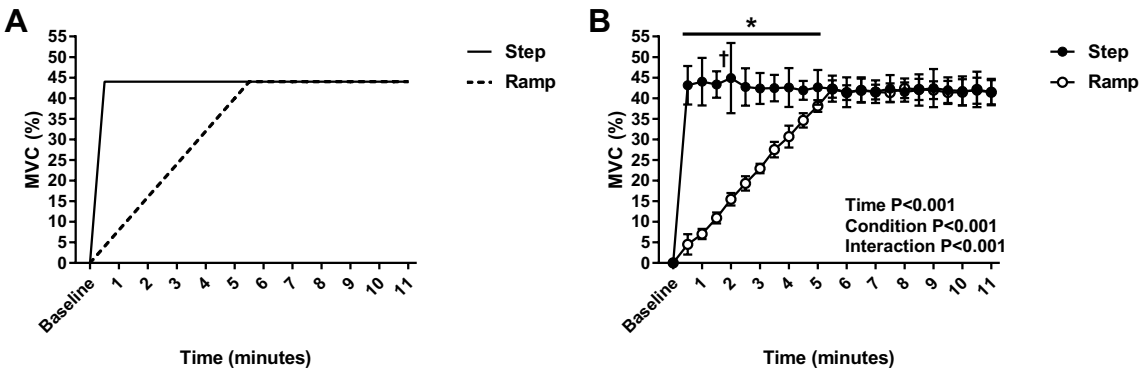


**Fig. 1** Illustration of the ramp and step protocols. **a** depicts the targeted ramp and step-increases in maximum voluntary contraction (MVC), and **b** displays the actual ramp (open circles) and step (filled circles) increases in MVC. In the ramp protocol (dotted line panel **a**), participants began performing rhythmic handgrip exercise at 4% of MVC, before incrementally increasing MVC by 4% every 30 s for 5.5 min, until 44% MVC was attained for the remainder of the protocol (an additional 5.5 min). In the step protocol (solid line panel **a**), participants performed rhythmic handgrip exercise at 44% MVC for the entirety of the protocol (11 min). Data are presented as mean ± SD. *$P < 0.05$ step versus ramp protocols; †$P < 0.05$ min 2 versus minutes 4.5, 6, 6.5, 7, 8, 8.5, 9.5, 10, 10.5, and 11 in the step protocol

### Brachial artery diameter

Standardized software approaches to acquire and analyze the ultrasound recordings were employed as previously described (Pyke et al. 2008a). A region of interest was placed around the highest quality portion of the B-mode longitudinal image of the artery. The software automatically and continuously tracks the walls of the vessel within the region of interest at a frequency of 30 Hz (Encoder FMD & Bloodflow v3.0.3, Reed Electronics, Australia) (Woodman et al. 2001). The diameter data were averaged into 3-s time bins.

### Brachial artery shear rate

Three second average time bins of mean blood velocity were analyzed offline using data acquisition software (LabChart, ADInstruments) (Pyke et al. 2008a). Mean shear rate was calculated as 4*blood velocity / brachial artery diameter (Gnasso et al. 2001) and reported for baseline and each minute of handgrip exercise (minutes 1–11).

### SS-FMD

SS-FMD was calculated as the absolute (mm) and relative (%) change from baseline to the average diameter recorded during each minute of handgrip exercise (minutes 1–11).

### Statistics

All statistical analyses were performed using IBM SPSS 24 (International Business Machines Corp, USA). Data were compared within-participants with significance set at $P < 0.05$ and are presented as mean ± SD. Data were analyzed using a linear mixed model with a compound symmetry co-variance structure. Two-factor linear mixed models were performed with time (repeated variable; minutes 1–11) and condition (repeated variable; step versus ramp protocol) for SS-FMD (relative and absolute), shear rate, %MVC, MAP, and HR. To identify the SS-FMD threshold (i.e. point of initial increase in diameter from baseline) in the ramp condition, one-factor linear mixed modelling was performed on arterial diameter with time as the factor (repeated variable; minutes 0–11). When significant main effects were detected, Bonferroni-corrected post-hoc tests were used to make pairwise comparisons. The same linear mixed models were performed for SS-FMD with shear rate included as a covariate. To test for agreement between trials, the intraclass correlation coefficient was calculated for minute 11 of SS-FMD in the step and ramp conditions. Further, Pearson correlations were performed between minute 11 SS-FMD (%) in the step and ramp protocols. The slope of SS-FMD increases during the period of steady-state shear rate in the step (minutes 2–11) and ramp (minutes 7–11) conditions was compared using paired t-tests; Pearson correlation was also performed on these slopes. Finally, a Pearson correlation was performed to identify the relationship between SS-FMD in minute 2 ("initial dilation") and the minute 2–11 dilation slope ("continuing dilation") within the step protocol. Baseline parameters (baseline diameter, baseline mean shear rate, and HR) were also compared between conditions (step versus ramp protocols) using paired *t* tests.

## Results

### Baseline parameters

Baseline parameters and *P* values are presented in Table 1. Baseline HR, diameter, and mean shear rate were similar between conditions.

### Ramp and step protocol MVC

Handgrip intensity for the step and ramp protocols is presented in Fig. 1b. In the ramp protocol, MVC rose progressively for 5 min before reaching a steady-state at 5.5 min (targeted 44%; actual 42 ± 2%). By contrast, the step protocol achieved a steady-state at 0.5 min (43 ± 5%), although MVC at minute 2 was slightly greater than several other 30-s intervals (Fig. 1b). MVC was greater in the step protocol than the ramp protocol until 5.5 min; thereafter, MVC was not different between conditions.

### Ramp and step protocol shear rate

Shear rate during the ramp and step protocols are displayed in Fig. 2a. In the ramp protocol, shear rate rose progressively before reaching a steady-state at minute 6. By contrast, shear rate in the step protocol was similar throughout handgrip exercise. Shear rate was lower in the ramp protocol compared to the step protocol from minutes 1–6; from minute 7 onward, shear rate was not different between conditions (minute 7, $P = 0.264$; minute 8, $P = 0.514$; minute 9, $P = 0.971$; minute 10, $P = 0.218$; minute 11, $P = 0.537$).

**Table 1** Baseline parameters for the step and ramp protocols

| | Step | Ramp | *P* value |
|---|---|---|---|
| Heart rate ($min^{-1}$) | 64 ± 9 | 64 ± 7 | 0.365 |
| Baseline diameter (mm) | 3.71 ± 0.28 | 3.75 ± 0.27 | 0.131 |
| Mean shear rate ($s^{-1}$) | 96 ± 43 | 103 ± 50 | 0.262 |

Data are presented as mean ± SD

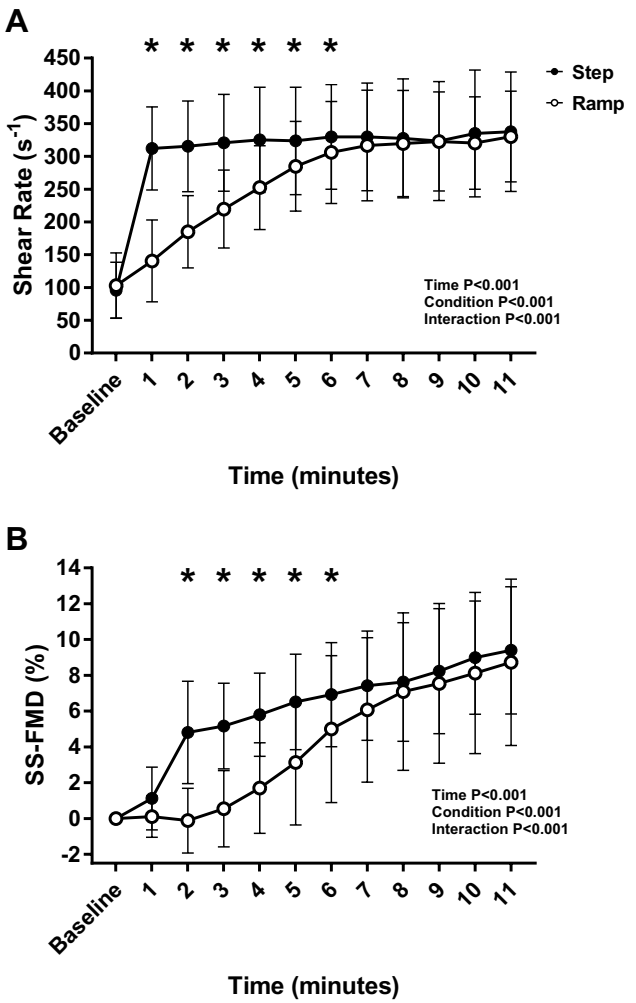


**Fig. 2** Shear rate (panel **a**) and flow-mediated dilation stimulated by sustained increases in shear stress (SS-FMD; panel **b**) in the ramp (open circles) and step (filled circles) protocols. Data are presented as mean ± SD. *$P < 0.05$ step versus ramp protocols

### Ramp and step protocol SS-FMD

Relative SS-FMD (%) is presented in Fig. 2b and absolute SS-FMD (mm) is presented in Table 2. SS-FMD (%) was greater during the step protocol compared to the ramp protocol from minutes 2–6. During minute 1, SS-FMD was not different between conditions ($P = 0.154$). SS-FMD was also not different between conditions during minutes 7 ($P = 0.057$), 8 ($P = 0.449$), 9 ($P = 0.335$), 10 ($P = 0.223$), and 11 ($P = 0.343$). These findings were similar for absolute SS-FMD. Including shear rate as a covariate did not alter interpretation. Individual SS-FMD during minute 11 of handgrip exercise during each protocol is displayed in Fig. 3. The intraclass correlation coefficient for SS-FMD during the last minute of exercise (minute 11) of each condition was 0.775 (95% confidence interval 0.502–0.909). SS-FMD in minute 11 correlated between step and ramp protocols ($r = 0.804$, $P < 0.001$). The order of protocols did not impact SS-FMD (minute 11: first test, 8.69 ± 3.84%; second test, 9.44 ± 4.41%; $P = 0.854$).

In the ramp protocol, significant vasodilation (i.e. increase in arterial diameter from baseline) was not present until the fifth minute of handgrip exercise ($P < 0.001$ min 5–11 versus baseline; mean shear rate in fifth minute = 285 ± 69 $s^{-1}$).

The slope of the increase in SS-FMD over time once steady-state shear rate was achieved did not differ between the ramp (minutes 7–11; 0.64 ± 0.57% $min^{-1}$) and step (minutes 2–11; 0.51 ± 0.36% $min^{-1}$) protocols ($P = 0.259$) and correlated between conditions ($r = 0.596$, $P = 0.009$). SS-FMD in minute 2 ("initial dilation") and the slope of the step protocol from minutes 2–11 ("continued dilation") were not correlated ($r = 0.210$, $P = 0.402$).

### Ramp and step protocol HR and MAP

The impact of the ramp and step protocols on systemic haemodynamic parameters are presented in Table 2. HR and MAP rose throughout the protocols. Although the interaction between time and condition did not reach significance (HR $P = 0.069$; MAP $P = 0.054$), visual inspection reveals that HR and MAP increased more abruptly at the onset of exercise in the step protocol and that values were similar between conditions in the latter half of the exercise bout when the intensity target (44% MVC) was the same.

## Discussion

The aim of this investigation was to assess the impact of the rate of increase in shear stress on SS-FMD. The primary finding is that SS-FMD was similar between ramp and step-increases in shear rate once the same shear rate magnitude was achieved. With maintenance of a stable shear rate, SS-FMD continued to increase over time in both conditions with a similar slope. This indicates that in humans, conduit artery SS-FMD is determined by the magnitude and duration, but not the rate, of the increase in shear stress. These findings contrast with observations in rat arterioles wherein a step-increase in shear stress results in a larger SS-FMD response versus a ramp for ~ 10 min following achievement of the same steady-state shear stress (Butler et al. 2000). Taken together this suggests that arterial vasodilation in response to sustained increases in shear stress differs between species and/or vascular beds.

### Impact of the rate of increase in shear stress on SS-FMD

Techniques to administer sustained increases in shear stress for SS-FMD assessment in humans are varied, and the rate

**Table 2** Minute-by-minute absolute flow-mediated dilation (FMD) and changes in systemic haemodynamic parameters during handgrip exercise

| | Time (minute) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Absolute FMD (mm) | | | | | | | | | | | |
| Step | 0.04 ± 0.07 | 0.18 ± 0.11† | 0.19 ± 0.10† | 0.22 ± 0.10† | 0.24 ± 0.11† | 0.26 ± 0.12† | 0.28 ± 0.13 | 0.28 ± 0.13 | 0.31 ± 0.14 | 0.34 ± 0.13 | 0.35 ± 0.14 |
| Ramp | 0.00 ± 0.04 | 0.00 ± 0.07 | 0.02 ± 0.08 | 0.07 ± 0.10 | 0.12 ± 0.14 | 0.19 ± 0.16 | 0.23 ± 0.16 | 0.27 ± 0.17 | 0.29 ± 0.17 | 0.31 ± 0.18 | 0.33 ± 0.18 |
| Time $P < 0.001$; Condition $P < 0.001$; Interaction $P < 0.001$ | | | | | | | | | | | |
| ΔHR (bpm) | | | | | | | | | | | |
| Step | 3.6 ± 4.4 | 3.5 ± 3.6 | 4.9 ± 5.1 | 4.7 ± 3.8 | 5.3 ± 3.3§ | 5.4 ± 4.0§ | 5.1 ± 4.3§ | 5.4 ± 5.3§ | 5.5 ± 4.8§ | 6.7 ± 4.9†† | 7.1 ± 4.8‖ |
| Ramp | 0.4 ± 3.9 | 1.7 ± 3.2 | 2.5 ± 3.8 | 4.1 ± 4.0 | 5.5 ± 5.1§ | 6.3 ± 3.8§ | 5.6 ± 3.3§ | 6.0 ± 3.5§ | 6.5 ± 4.6§ | 6.9 ± 3.4†† | 7.0 ± 4.2‖ |
| Time $P < 0.001$; Condition $P = 0.172$; Interaction $P = 0.069$ | | | | | | | | | | | |
| ΔMAP (mmHg) | | | | | | | | | | | |
| Step | 3.6 ± 3.2 | 4.4 ± 2.9 | 4.0 ± 3.8 | 5.3 ± 3.3‡ | 6.2 ± 3.7‡ | 6.5 ± 3.8†† | 6.1 ± 4.4†† | 6.5 ± 4.5†† | 6.2 ± 5.8†† | 6.5 ± 5.8†† | 7.0 ± 5.2‖ |
| Ramp | 0.6 ± 3.5 | 2.3 ± 3.2 | 3.3 ± 3.4 | 3.7 ± 4.0‡ | 4.3 ± 3.7‡ | 5.8 ± 4.3†† | 6.0 ± 5.0†† | 6.4 ± 5.2†† | 7.2 ± 4.9†† | 7.1 ± 5.0†† | 7.8 ± 4.4‖ |
| Time $P < 0.001$; Condition $P = 0.016$; Interaction $P = 0.054$ | | | | | | | | | | | |

Data are presented as mean ± SD

† $P < 0.05$ versus ramp

‡ $P < 0.05$ versus minute 1

§ $P < 0.05$ versus minute 1 and 2

†† $P < 0.05$ versus minute 1, 2, and 3

‖ $P < 0.05$ versus minute 1, 2, 3 and 4

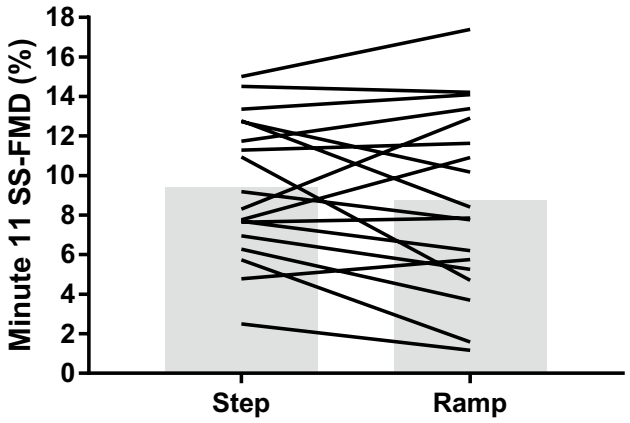


**Fig. 3** Individual flow-mediated dilation stimulated by sustained increases in shear stress (SS-FMD) during the final minute of handgrip exercise (minute 11) in the ramp and step protocols. The shaded bar depicts the mean for each condition

of increases in shear stress to steady-state may be immediate [i.e. step-increases (Pyke et al. 2008a, b, 2004)], of short duration [i.e. handgrip exercise (~ 30–120 s) (D'Urzo et al. 2018; Pyke et al. 2008b; Slattery et al. 2016; Szijgyarto et al. 2014; Pyke and Jazuli 2011; McPhee and Pyke 2018)], or of long duration [i.e. heating (~ 10–35 min) (Bellien et al. 2006; Joannides et al. 2002; Mullen et al. 2001; Savage and Brengelmann 1994)]. Whether studies with varying rate of increase in shear stress interrogate distinct mechanisms or provide similar information was unclear. Cell culture studies depict a biphasic nitric oxide production (Frangos et al. 1996). The first phase of nitric oxide production is responsive to the rate of increase in shear stress and is $Ca^{2+}$ and G-protein-dependent (Kuchan and Frangos 1994), whereas the second phase is responsive to the magnitude of the sustained increase in shear stress, and is kinase-dependent and $Ca^{2+}$ and G-protein-independent (Kuchan and Frangos 1994; Balligand et al. 2009). In isolated rat arterioles, administering a step-increase in shear stress elicited a greater SS-FMD than a 5-min ramp to the same steady-state even after 10 min of similar steady-state shear stress (Butler et al. 2000). The findings of the present investigation in human conduit arteries contrasts these previous reports in cell culture and isolated arteriole studies.

In human conduit arteries, we did not observe any impact of the rate of increase in shear stress on SS-FMD once a similar stimulus magnitude was achieved. Therefore, the influence of the rate of increase in shear stress may be species and/or vessel-dependent. The magnitude of SS-FMD in the brachial artery appears to reflect the steady-state increase in shear stress, rather than how quickly steady-state was attained. From a methodological perspective, this suggests that conduit artery SS-FMD elicited with techniques that vary in the rate of stimulus onset (e.g. exercise, heating) reflects similar shear stress transduction mechanisms and can be reasonably compared.

## Impact of the duration of the shear stress stimulus on SS-FMD

Despite the increase in shear stress remaining steady from minutes 2–11 in the step protocol and minutes 7–11 in the ramp protocol, SS-FMD continued to increase, indicating that the duration of the increase in shear stress also contributes to SS-FMD. This observation is in agreement with several previous human studies reporting a lack of plateau in SS-FMD despite maintenance of a steady-state shear stress stimulus (Pyke et al. 2008a, b; Slattery et al. 2016; Findlay et al. 2013). The continued gradual SS-FMD appears to be vulnerable to impairment as it was absent in young, otherwise healthy male smokers (plateau in SS-FMD after 2 min) (Findlay et al. 2013) while SS-FMD continued to increase during steady-state shear stress in healthy control participants. Evidence from *in vitro* and modelling studies suggests distinct phases of vasodilator production (Sriram et al. 2016; Balligand et al. 2009; Frangos et al. 1996; Kuchan and Frangos 1994), which may indicate that the continuing SS-FMD reflects distinct transduction and/or vasodilator pathways versus the initial SS-FMD. Supporting the potential for mechanistic distinction, in this investigation there was no correlation between the step condition "initial SS-FMD" (SS-FMD magnitude in minute 2) and the slope of the continued dilation during steady-state shear stress. The very similar SS-FMD between conditions from minutes 7–11 may indicate that any combination of mechanisms involved in the step condition were recruited incrementally during the ramp, reaching similar recruitment upon achieving the same steady-state shear stress magnitude.

## Identification of a threshold stimulus for SS-FMD

The ramp protocol permitted the identification of a shear stress threshold necessary to elicit conduit artery vasodilation. Arterial diameter significantly increased from baseline at the fifth minute of handgrip exercise during the ramp protocol, when shear rate was $285 \pm 69\ s^{-1}$. This aligns with a previous report that identified a vasodilation threshold of approximately $250\ s^{-1}$ in the human brachial artery (Wray et al. 2011). Similarly, a vasodilation threshold has been reported in the iliac artery of anesthetized pigs (Kelly and Snow 2007). Thus, conduit arteries appear to possess a shear stress threshold for vasodilation to occur. Whether this threshold changes with age or disease, and how it relates to other aspects of endothelial function is unknown.

### Methodological considerations

The present investigation was performed only in the brachial artery of young, healthy males and thus cannot be generalized to other vessels or populations. Further, we only compared two rates of increase in shear stress: an immediate step-increase, and a gradual 5.5-min ramped-increase. However, despite the prolonged ramp-increase, both shear rate and SS-FMD were similar once steady-state was achieved. These findings agree with a previous investigation of step versus uncontrolled (~60 s to steady-state) handgrip exercise induced increases in shear stress (Pyke et al. 2008b). Thus, across a range of rates of increase in shear stress, SS-FMD appears to reflect the magnitude and duration of the sustained stimulus.

## Conclusion

The primary objective of this study was to determine the influence of the rate of increase in shear stress on SS-FMD. We created step and ramp increases in shear stress to create immediate and gradual (5.5 min) rates of increase to a similar steady-state shear stress stimulus. We observed a similar, gradually increasing SS-FMD magnitude once steady-state increases in shear stress were attained. These findings suggest that in the brachial arteries of young, healthy males, SS-FMD magnitude reflects the magnitude and duration of the increase in shear stress, regardless of its initial rate of increase. These findings contrast with observations in animal arterioles and further research is required to compare conduit and arteriolar responses in humans. From a methodological perspective, when seeking to investigate human conduit artery endothelial function, variability in the rate of increase in shear stress does not appear to confound comparison of SS-FMD responses stimulated by a similar duration and magnitude of shear stress.

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**Author contributions** KEP and JSW conceived and designed the research. JSW and JCT performed the data collection and analysis. JCT drafted the manuscript. KEP and JSW revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript for submission.

### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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