Rapid vasoregulatory mechanisms in exercising human skeletal muscle: dynamic response to repeated changes in contraction intensity

Anna M. Rogers, Natasha R. Saunders, Kyra E. Pyke, and Michael E. Tschakovsky

Human Vascular Control Laboratory, School of Physical and Health Education, Queen’s University, Kingston, Ontario, Canada

Submitted 7 April 2006; accepted in final form 26 April 2006

Rogers, Anna M., Natasha R. Saunders, Kyra E. Pyke, and Michael E. Tschakovsky. Rapid vasoregulatory mechanisms in exercising human skeletal muscle: dynamic response to repeated changes in contraction intensity. Am J Physiol Heart Circ Physiol 291: H1065–H1073, 2006.—We tested the hypothesis that vasoregulatory mechanisms exist in humans that can rapidly adjust muscle blood flow to repeated increases and decreases in exercise intensity. Six men and seven women (age, 24.4 ± 1.3 yr) performed continuous dynamic forearm handgrip contractions (1- to 2-s contraction-to-relaxation duty cycle) during repeated step increases and decreases in contraction intensity. Three step change oscillation protocols were examined: Slow (7 contractions per contraction intensity × 10 steps); Fast (2 contractions per contraction intensity × 15 steps); and Very Fast (1 contraction per contraction intensity × 15 steps). Forearm blood flow (FBF; Doppler and echo ultrasonography), heart rate (ECG), and mean arterial pressure (arterial tonometry) were examined for the equivalent of a cardiac cycle during each relaxation phase (FBF_relax). Mean arterial pressure and heart rate did not change during repeated step changes (P = 0.352 and P = 0.190). For both Slow and Fast conditions, relaxation phase FBF_relax adjusted immediately and repeatedly to both increases and decreases in contraction intensity, and the magnitude and time course of FBF_relax changes were virtually identical. For the Very Fast condition, FBF_relax increased with the first contraction and thereafter slowly increased over the course of repeated contraction intensity oscillations. We conclude that vasoregulatory mechanisms exist in human skeletal muscle that are capable of rapidly and repeatedly adjusting muscle blood flow with ongoing step changes in contraction intensity. Importantly, they demonstrate symmetry in response magnitude and time course with increasing versus decreasing contraction intensity but cannot adjust to very fast exercise intensity oscillations.

exercise hyperemia; muscle pump; rapid vasodilation; blood flow dynamics

THE ONSET OF EXERCISE IN HUMANS is characterized by an immediate increase in skeletal muscle blood flow that plateaus by 5–7 s (phase I) and is followed by a delayed, slower increase to steady-state levels starting at ~20 s (16, 19) (phase II). The initial immediate increase was for some time attributed solely to the muscle pump (14). However, in recent years, we have shown in humans (11, 18, 20) and others with in situ animal models (4, 6, 21) that resistance vessels can dilate immediately after the first contraction at exercise onset. It is currently unclear what mechanism(s) are responsible for this rapid vasodilation. VanTeeffelen and Segal (21) recently presented evidence for acetylcholine-mediated ascending dilation in hamster retractor muscle. They hypothesize that acetylcholine spillover from motor end plates is the mechanism for rapid vasodilation with contraction onset. In contrast, previous investigations in dogs (9) and humans (3), using stimulated and attempted motor nerve recruitment, respectively, during neuromuscular blockade of skeletal muscle, observed no increase in blood flow. This argued against spillover of motor end plate acetylcholine as a mediator of immediate dilation in larger muscles. Recently, we have proposed that mechanical distortion of resistance vessels with contraction directly affects smooth muscle (18) tone, potentially but not necessarily reflecting a myogenic reflex with decreased transmural pressure during contraction (7). Hamann et al. (4) have suggested endothelial release of vasodilator substance(s) with endothelial cell distortion. Recent work by Clifford et al. (2) has confirmed a mechanical distortion effect in isolated vessels that in part requires an intact endothelium.

Work in our laboratory (11) has identified that these rapid vasodilatory mechanism(s) are not just activated with the onset of muscle contraction but also with further increases in contraction intensity in human forearm exercise. Furthermore, they do not appear to be dependent on nitric oxide and prostaglandins because combined blockade does not affect the exercise-to-exercise rapid blood flow increase (10). In contrast, observations using repeated sinusoidal oscillations or single step changes of treadmill grade in rats and dogs argue that vasoregulation responses to changes in exercise intensity are sluggish (15). In addition, the lag in blood flow response to sinusoidal oscillations demonstrated a frequency dependence, whereby in rapid oscillations, the blood flow was highest when the workload was lowest and vice versa. Interestingly, the dynamics (rate of increase or decrease) of blood flow responses appeared to be temporally similar between increases and decreases in workload, although these investigators did not report on this. This study remains the only study that provides data on the temporal response of vasoregulation to both increases and decreases in exercise intensity.

With this as a background, we investigated the nature of vasoregulatory responses to repeated step increase/decrease oscillations in human muscle contraction intensity. We had subjects perform continuous rhythmic, dynamic forearm handgrip exercise during repeated step oscillations in contraction intensity. We did so under three different step oscillation frequencies. We hypothesized that, in the human forearm, rapid vasoregulatory mechanisms exist that are symmetrical in their responsiveness to increases and decreases in exercise intensity. Furthermore, we hypothesized that the maintenance...
of this symmetry may be dependent on the frequency of exercise intensity oscillations.

METHODS

Subjects

Thirteen healthy, nonsmoking men \( (n = 6) \) and women \( (n = 7) \) were recruited. Table 1 lists the characteristics of the subjects. The study was reviewed and approved by the Health Sciences Human Research Ethics Board of Queen’s University (Kingston, ON, Canada), which operates under the terms of the Declaration of Helsinki. Subjects were required to fill out a medical questionnaire assessing cardiovascular health before participation, and after receiving a detailed description of the experimental protocol, they signed a consent form. The subjects were also prescreened to determine whether forearm anatomy allowed for an acceptable Doppler ultrasound signal from a vein.

Data Acquisition

Measurement of hemodynamic responses. Subjects lay supine with both arms out to the side at heart level. They were instrumented for beat-by-beat measurements as follows.

Heart rate was obtained via standard CM5 lead placement of ECG electrodes. Mean arterial blood pressure was measured on the contralateral arm supported at heart level via arterial tonometry (Colin 7000, Trudell Medical, London, ON, Canada).

Brachial artery mean blood flow velocity (MBV) was measured with pulsed Doppler velocimetry (Multigon 500B Transcranial Doppler, Multigon Industries, Yonkers, NY) as described in previous work \( (11, 18, 20) \). These measures were performed continuously and collected on a personal computer data acquisition system (Chart 4.0, ADInstruments, Colorado Springs, CO) at 200 Hz.

Brachial artery diameter measurements were performed immediately proximal to the site of the MBV measurements with the use of Echo Ultrasonography (Vingmed System 5, GE Medical Systems). Briefly, a 10-MHz linear probe operating in two-dimensional B-mode was positioned over the brachial artery, and a clear image of the vessel was obtained and recorded to videotape at the following times: 1) three times at rest during the minute preceding exercise, 2) at 3 min 30 s of steady-state, light-weight (LW) exercise, 3) immediately after the first step increase and decrease in each trial, 4) at the halfway point in the step increases and decreases in each trial, 5) immediately after the final step increase and decrease in contraction intensity (see Fig. 1 for protocol). Brachial artery diameter was later measured from the videotaped ultrasound images as follows: On-screen calipers were manually placed on a clear section of the vessel wall at three points on a frozen screen image of the brachial artery during diastole and then averaged to estimate the vessel diameter for that time interval. All diameter measures were made by the same operator. Ultimately, there was virtually no change in diameter across each set of step changes, so a single diameter value averaged across these measurement time points was used.

Forearm blood flow (FBF) was calculated as \( \text{FBF} = \text{MBV} \times 60 \times \text{MBV} \times 60 \times \text{MBV} \times 60 \) s·min⁻¹·m⁻²·(brachial artery diameter/2)², where FBF is in milliliters per minute, MBV is in centimeters per second, and the brachial artery diameter is in centimeters. FBF across contraction/relaxation cycles represents the combined effect of muscle contraction-induced mechanical impedance and enhancement of blood flow and vascular conductance. To isolate vasoregulatory responses to the changes in contraction intensity, we quantified the FBF during the equivalent of a cardiac cycle in the relaxation phase between contractions \( \text{FBF}_{\text{relax}} \) (see Fig. 2). Because transitions were between different contraction intensities, there was no addition or subtraction of muscle pump flow enhancement \( (11) \). Additionally, when mean arterial pressure is taken

![Fig. 1. A: schematic of forearm contraction intensity step changes. Solid squares indicate timing of a 1-s contraction separated by a 2-s rest period. For the very fast step change frequency, single contractions at alternating weights were performed. For the fast step change frequency, two contractions at alternating weights were performed. For the slow step change frequency, 7 contractions at alternating weights were performed. B: schematic illustration of timing of baseline light weight (LW) exercise and the three sets of repeated step changes (10 repeats for slow step change frequency and 15 repeats for fast and very fast step change frequency). MW, moderate weight.](http://ajpheart.physiology.org/)

- Values are means ± SE; \( n \), number of subjects.
into account and the response is expressed as forearm vascular conductance (data not shown), it is virtually identical to $FBF_{relax}$. This is not surprising, as we observed no changes in blood pressure across repeated step changes in contraction frequency (see Table 3). Therefore, measures immediately upon relaxation were taken to represent changes in $FBF_{relax}$ due to changes in resistance vessel caliber (i.e., relative vasodilation and vasoconstriction).

The experiments were performed in a cool environment (room temperature 18°–20°C), and the forearm and hand were cooled in subjects by using temporary application of an ice pack when necessary before the onset of experiments to ensure minimal resting FBF to the skin (17). The ice pack was removed at least 5 min before the onset of any exercise, and resting FBF was confirmed to remain minimal during this time.

**Experimental Design**

**Forearm exercise.** Forearm exercise consisted of rhythmic, dynamic handgrip contractions (1- to 2-s contraction-to-relaxation duty cycle) in which a weight suspended over a pulley was lifted and lowered over a 5-cm distance during the 1-s contraction period. Complete relaxation occurred for 2 s between each contraction. The men exercised with a LW of 10 lb. and a moderate weight (MW) of 20 lb. The women alternated between a LW of 8 lb. and a MW of 16 lb. The weights corresponded to 10% (LW) and 20% (MW) of the maximal voluntary contraction for this group of men and women.

**Protocols for repeated manipulation of contraction intensity.** Figure 1 illustrates the exercise protocols consisting of step increases and decreases in contraction intensity at different frequencies. After 4 min of LW exercise to establish steady state, repeated step oscillations in weight from LW to MW began (a step oscillation refers to a complete cycle of weight increase and decrease). Three step change oscillation conditions were examined (see Fig. 1): 1) Very Fast oscillations (1 to 1): one contraction per contraction intensity period; 2) Fast oscillations (2 to 2): two contractions per contraction intensity period; and 3) Slow oscillations (7 to 7): seven contractions per contraction intensity period.

Subjects were instrumented and instructed to lie quietly to ensure that blood velocity was at a baseline level. After 1 min of recording at rest, the subject began 4 min of LW exercise to reach a steady-state blood flow. Weight was then repeatedly added and removed until a total of 15 step oscillations (1 set) had occurred for the 1-to-1 and 2-to-2 trials (see Fig. 1), and a total of 10 step oscillations (1 set) had occurred for the 7-to-7 trial (see Fig. 1). One trial consisted of three sets of step oscillations. The sets were separated by 3 min of steady-state LW exercise to allow blood flow to normalize to a steady state before initiation of the next set. Each trial was separated by 15 min of rest. The order of 1-to-1, 2-to-2, and 7-to-7 step oscillations was counterbalanced between subjects to eliminate any order effect.

The contraction intensity and oscillation manipulation approach utilized in this study was based on the following rationale. From previous data in our laboratory (11), we determined that forearm veins are maximally emptied at light contraction intensities, indicating that the forearm muscle pump is completely engaged below 10% maximal voluntary contraction intensity in this exercise model and that it does not contribute to increasing blood flow to exercising muscle in an exercise-to-exercise transition. Furthermore, the rationale for using exercise-to-exercise transitions, while maintaining a constant contraction speed (i.e., treadmill speed to maintain muscle pump frequency), was discussed previously (13, 15) as a means of examining vasoregulatory changes with exercise intensity changes in isolation of the muscle pump.

Initial rapid vasoregulatory adjustments to increased exercise intensity plateau at ~5–7 s (phase I), and a second slower component (phase II) begins at ~20 s (16, 19). Therefore, with a change in the contraction intensity at every contraction (very fast frequency) and every two contractions (fast frequency), blood flow was challenged to adapt to a new exercise intensity during what would have been the initial adjustment (1 to 1) and just before (2 to 2) the phase I plateau is reached. The low-frequency step change occurred at what would have been the end of phase I. We reasoned that these rapid vasoregulatory adjustments might be least responsive to reductions in contraction intensity if occurring before a plateau was reached (very fast and fast frequencies) and more responsive if contraction intensity changed once the plateau had been well established.

**Data Analysis**

As mentioned previously, the equivalent of a single cardiac cycle unaffected by contraction during each relaxation period between contractions (see Fig. 2) was analyzed. The data for each subject were averaged across the three sets for each oscillation condition trial to yield a single subject response characterization. This provided a profile of vasoregulatory responses across repeated step changes in...
Statistical Analysis

Heart rate and mean arterial pressure responses. Two-way, repeated-measures ANOVA was used to compare heart rate and mean arterial pressure at baseline, steady state, and selected time points during the repeated step changes. The factors were step oscillation frequency (very fast, fast, and slow) and time (baseline, MW steady state, and selected time points during the repeated steps). Each step increase and each step decrease in contraction intensity was consistent with repeated step changes from LW to MW.

FBFrelax responsiveness to continuous step changes. Within each step oscillation frequency, a one-way, repeated-measures ANOVA was used to test the hypothesis that the magnitude of immediate FBFrelax change in the first relaxation after a change in exercise intensity was consistent with repeated step changes from LW to MW.

Symmetry of FBFrelax to increase and decrease in exercise intensity. Two-way, repeated-measures ANOVA was used to determine whether the absolute change in FBFrelax was the same following a step increase versus step decrease in exercise intensity between slow and fast step change frequencies. The factors were step oscillation frequency (slow, fast, and very fast) and relaxation number (first or second).

Table 2. Heart rate response to repeated step increases and decreases in contraction intensity

<table>
<thead>
<tr>
<th>Step Change Frequency</th>
<th>Resting Baseline</th>
<th>Light-Weight Steady State</th>
<th>First Step Weight Increase</th>
<th>First Step Weight Decrease</th>
<th>Final Step Weight Increase</th>
<th>Final Step Weight Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow steps</td>
<td>63.2±2.7</td>
<td>64.5±1.7</td>
<td>64.8±2.2</td>
<td>64.4±1.8</td>
<td>64.7±1.7</td>
<td>62.6±1.9</td>
</tr>
<tr>
<td>Fast steps</td>
<td>62.5±3.3</td>
<td>62.5±2.2</td>
<td>63.3±2.6</td>
<td>62.7±2.6</td>
<td>62.9±2.1</td>
<td>62.7±2.1</td>
</tr>
<tr>
<td>Very fast steps</td>
<td>59.7±2.0</td>
<td>61.4±2.0</td>
<td>62.8±2.4</td>
<td>61.3±2.3</td>
<td>61.8±1.8</td>
<td>60.7±1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for heart rate (HR; in beats/min). Resting baseline, HR before start of exercise; light-weight steady state, HR at end of 4 min of initial light-weight exercise; first step weight increase, HR during first step increase from light to moderate weight in the set of repeated steps; first step weight decrease, HR during first step decrease from moderate to light weight in the set of repeated steps; final step weight increase, HR during final step increase from light to moderate weight in the set of repeated steps; and final step weight decrease, HR during final step decrease from moderate to light weight in the set of repeated steps.

For all tests, post hoc analysis was performed using Tukey’s honestly significant difference technique. *P < 0.05 was used to attribute statistical significance. All data are reported as means ± SE.

RESULTS

Heart Rate and Mean Arterial Pressure Response

Tables 2 and 3 summarize the heart rate and mean arterial pressure responses over the course of repeated step oscillations in contraction intensity. There was no change in heart rate with the onset of LW contractions. There was no change in heart rate with the onset of step oscillations in any of the step oscillation trials (P = 0.19). There was no difference in heart rate between step oscillation frequencies (P = 0.352).

Mean arterial pressure did not change with the onset of LW exercise relative to resting baseline (P = 0.682). However, it was elevated versus resting baseline with the first step increase in contraction intensity for all three step change frequencies (P = 0.023). Thereafter, it was not different when measured at the final step change in contraction intensity (P = 0.352). Furthermore, it did not differ between step oscillation frequencies (P = 0.287).

FBFrelax Response to Repeated Step Oscillations in Contraction Intensity

Slow oscillations (7 to 7). Figure 3 illustrates the FBFrelax profile to repeated step oscillations in contraction intensity, where contraction intensity was changed every seven contractions. Each step increase and each step decrease in contraction intensity resulted in an immediate respective increase and decrease in FBFrelax (all P < 0.05). This immediate change was followed by continued progressive change in FBFrelax until the next contraction intensity change. The profile of an FBFrelax increase with a repeated contraction intensity increase was similar across the 2nd through 10th step oscillation (P = 0.581) for increase after first MW, and P = 0.114 for increase by...
seventh contraction). These were therefore averaged to yield a single step oscillation profile for analysis of symmetry in the FBF_{relax} response to increased versus decreased contraction intensity (see Symmetry of FBF_{relax} Increase and Decrease With Respective Contraction Intensity Changes).

**Fast oscillations (2 to 2).** Figure 4 illustrates the FBF_{relax} profile to repeated step oscillations in contraction intensity, where contraction intensity was changed every two contractions. Each step increase and each step decrease in contraction intensity resulted in an immediate respective increase and decrease in FBF_{relax}. This immediate change was followed by a further change in FBF_{relax} with the second contraction at the new contraction intensity. The profile of FBF_{relax} increase and decrease with contraction intensity increase and decrease was similar across step oscillations 2–15 ($P = 0.684$ for increase following first MW, and $P = 0.291$ for increase by 2nd contraction of MW). These were therefore averaged to yield a single step oscillation profile for analysis of symmetry in the FBF_{relax} response to increased versus decreased contraction intensity (see Symmetry of FBF_{relax} Increase and Decrease With Respective Contraction Intensity Changes).

**Very fast oscillations (1 to 1).** Figure 5 illustrates the FBF_{relax} profile to repeated step oscillations in contraction intensity, where contraction intensity was changed every contraction. The initial step increase resulted in an immediate increase in FBF_{relax} to a level that was virtually identical to that for the other step oscillation frequency trials (in ml/min; 1 to 1: $269.2 \pm 14.8$; 2 to 2: $278.7 \pm 23.2$; and 7 to 7: $276.7 \pm 17.4$) ($P = 0.923$). However, unlike the slow and fast conditions, there were no reductions in FBF_{relax} with single contraction reductions in contraction intensity. Instead, FBF_{relax} increased gradually over time until approximately the 15th–19th contraction, at which point no further increases occurred. Thus there was no evidence for immediate adjustments in FBF_{relax} with contraction intensity oscillations as had occurred in the slow and fast oscillation trials.

**Symmetry of FBF_{relax} Increase and Decrease With Respective Contraction Intensity Changes**

Because there were no differences between individual step oscillations within each set of fast and slow step oscillations,
we averaged these together (2nd to 10th for slow, 2nd to 15th for fast) to examine the symmetry of the FBFrelax response to an increase versus decrease in contraction intensity. Figure 6, A–D, illustrates the average step change profile of FBFrelax for the slow and fast oscillation trials and the remarkable symmetry that is apparent. For the slow oscillation trials, there was no difference between the response to an increase versus decrease in contraction intensity (main effect of contraction intensity direction change: \( P = 0.269 \)). The same was the case for the fast oscillation trials (main effect of contraction intensity direction change: \( P = 0.411 \)). Figure 7 provides further insight into this symmetry. The magnitude of change of FBFrelax with increases versus decreases in contraction intensity was not different in slow versus fast step oscillation frequency (main effect of step oscillation frequency: \( P = 0.809 \); interaction effect of contraction number \( \times \) step oscillation frequency: \( P = 0.209 \)).

**DISCUSSION**

This study investigated the nature of rapid blood flow regulatory responses with repeated step oscillations in contraction intensity in human muscle. We tested the hypotheses 1) that rapid vasoregulatory mechanisms exist in the human forearm that are symmetrical in their responsiveness to increases and decreases in exercise intensity and 2) that the maintenance of this symmetry may be dependent on the frequency of exercise intensity oscillations.

The major novel findings are in agreement with our hypotheses and are as follows: First, rapid vasoregulatory mechanisms exist that are capable of immediate, repeated responsiveness to repeated step oscillations in contraction intensity. Second, these mechanisms are unable to respond rapidly when contraction intensity is alternated every contraction but demonstrate rapid responsiveness when oscillations had two or more contractions per contraction intensity (i.e., they demonstrate a “frequency sensitivity”). Third, these mechanisms demonstrate a remarkable symmetry in their response to increases versus decreases in contraction intensity. Finally, this symmetry is identical in fast versus slow contraction intensity step oscillation frequencies.

**Rapid Response of FBFrelax: Vasoregulation or Muscle Pump?**

The observation that FBFrelax rapidly (with the first contraction of a contraction intensity change) responded to both increases and decreases in contraction intensity could suggest a mechanical muscle pump effect. However, there are a number of points that argue strongly against this in our experimental model and instead point to rapid vasoregulation. First, Sheriff and Zidon (15) have previously used a contraction intensity change versus contraction frequency change model to isolate mechanical muscle pump effects from vasoregulatory responses to step and sine wave changes in exercise intensity in a rat treadmill model. They found very sluggish responses to changes in contraction intensity versus rapid responses to changes in frequency. Second, we have demonstrated that emptying of forearm veins is already maximal with contractions below the 10% maximal voluntary contraction range (18) and have used this model of changes in contraction intensity previously to demonstrate (15) the existence of rapid vasodilatory mechanisms in exercise-to-exercise transitions in the human forearm (11). Finally, direct evidence from the very fast step oscillations in the current study further confirms that a muscle pump effect was not occurring with oscillations in contraction intensity. Figure 5 illustrates how FBFrelax responds rapidly only to the first step increase in contraction intensity. Thereafter, no rapid response of FBFrelax is evident with alternate light and moderate contraction intensity oscillations with each contraction. In the current study then, flow immediately after contraction (i.e., in the relaxation phase between contraction) is not being altered by intensity-of-contraction effects on the arterial-to-venous pressure gradient.

There were no changes in mean arterial pressure during step changes in contraction intensity (see Table 3). Furthermore, analysis of data taking into account arterial blood pressure to calculate forearm vascular conductance demonstrated the same response characteristics and magnitudes as FBFrelax. Thus we report FBFrelax adjustments with changes in contraction intensity in this forearm exercise model and interpret them to reflect
exclusively changes in resistance vessel caliber due to active vasoregulatory mechanisms.

**Insight Into Nature of Rapid Vasoregulatory Mechanisms**

Maintained responsiveness to repeated step changes in contraction intensity. Sheriff and Zidon (15) were the first to examine exercising limb blood flow responses to oscillations in exercise intensity. Their model utilized rapid step increase in treadmill grade from $10\%$ to $10\%$ versus rapid step increase in treadmill speed. They observed that there was an $-5$-s delay before the onset of blood flow increases with a step increase in treadmill grade. This delay was even longer when the treadmill grade oscillated in a continuous sinusoidal pattern. However, the oscillations in blood flow with this repeated oscillation in treadmill grade were maintained. These data indicated that vasoregulatory mechanisms are sluggish in their onset response with changes in exercise intensity.

In contrast, we have consistently demonstrated that FBF_{relax} increases immediately with a step change in forearm contraction intensity (10, 11). Recently, data from isolated dog muscles (4) and from intravital videomicroscopy of rodent muscle (6, 21) have confirmed that resistance vessels dilate virtually exclusively changes in resistance vessel caliber due to active vasoregulatory mechanisms.

![Fig. 6. A: profile of FBF_{relax} averaged over repeated step changes in contraction intensity for slow step change frequency condition. First data point represents FBF_{relax} for the last LW contraction preceding the increase in contraction intensity; 8th data point represents FBF_{relax} for the last MW contraction preceding the decrease in contraction intensity. Solid arrow and dashed arrow identify response to the increase and decrease in contraction intensity, respectively. B: absolute change in FBF_{relax} with a change in contraction intensity ($\Delta$, increase in contraction intensity; $\bullet$, decrease in contraction intensity). Solid and dashed arrows indicate how data from A have been reoriented for comparison of temporal and magnitude characteristics of FBF_{relax} response to increase vs. decrease in contraction intensity. C: profile of FBF_{relax} averaged over repeated step changes in contraction intensity for fast step change frequency condition. First data point represents FBF_{relax} for the last LW contraction preceding increase in contraction intensity; 3rd data point represents FBF_{relax} for the last MW contraction preceding decrease in contraction intensity. Solid arrow and dashed arrow identify response to increase and decrease in contraction intensity, respectively. D: absolute change in FBF_{relax} with a change in contraction intensity ($\Delta$, increase in contraction intensity; $\bullet$, decrease in contraction intensity). Solid and dashed arrows indicate how data from C have been reoriented for comparison of temporal and magnitude characteristics of FBF_{relax} response to increase vs. decrease in contraction intensity. A and C: *significantly elevated compared with last contraction at LW contraction intensity ($P < 0.05$); #significantly reduced compared with last contraction at MW contraction intensity ($P < 0.05$).](http://ajpheart.physiology.org/)

![Fig. 7. Absolute change in FBF_{relax} data from Fig. 6 to allow comparison of this response with first two contractions of a contraction intensity decrease and increase between slow and fast step change frequency conditions. For slow (7 to 7) step change oscillation: $\circ$, change in FBF with increase in contraction intensity; $\bullet$, change in FBF with decrease in contraction intensity. For fast (2 to 2) step change oscillation: $\square$, change in FBF with increase in contraction intensity; $\bullet$, change in FBF with decrease in contraction intensity. No differences between step change oscillation frequencies at contraction 1 or 2; $P = $ not significant (see RESULTS).](http://ajpheart.physiology.org/)
immediately at the onset of exercise. In the present study, we observed the characteristic immediate increase in relaxation FBF_{relax} after the first contraction of a step change in exercise intensity (see Figs. 3–5). Differences between the treadmill and forearm studies may be explained by the very small and gradual changes in contraction intensity with rat treadmill exercise versus immediate and substantial changes in forearm contraction intensity.

The results of this study add to our previous work by demonstrating repeated rapid increases and decreases in FBF_{relax} with contraction intensity step oscillations. We believe our data most likely reflect increases and decreases in vasodilator influence, rather than competing vasodilator and active vasoconstrictor influences. Currently, the only vasodilator that appears capable of evoking rapid vasodilation in vivo would be skeletal muscle-released K^+ (1, 8), because motor end plate acetylcholine release only seems functional in hamster cremaster muscle preparations (21). Alternately, it has been proposed that mechanical distortion of resistance vessel smooth muscle and/or endothelium (4, 18, 19) might lead to dilatation.

Smooth muscle tone is primarily dependent on cytosolic Ca^{2+} concentration ([Ca^{2+}]_i) and to a lesser degree on Ca^{2+} sensitivity (5). The observation that FBF_{relax} can rapidly and repeatedly respond to repeated contraction intensity changes indicates that if a vasodilatory substance is involved, its interstitial concentration and its effect on smooth muscle cytosolic [Ca^{2+}] must be able to increase and decrease rapidly. The study by Wunsch et al. (22) is the only one we are aware of that has documented the time delay between exposure of a resistance vessel to putative vasodilator substances and vasodilation. These investigators observed a 4- to 5-s delay before the onset of vasodilation when acetylcholine, K^+, or sodium nitroprusside (nitric oxide donor) was directly applied to an isolated resistance vessel via micropipette. It is not currently possible to examine interstitial concentrations of vasodilator with the temporal resolution to adequately evaluate interstitial oscillations as a potential explanation for our observations. Certainly, such oscillations would not be consistent with vasodilator substance production/release and blood flow washout determining interstitial concentration (“metabolic” hypothesis; see Ref. 12).

In contrast to interstitial vasodilator appearance, a mechanical distortion mechanism would not be dependent on rapid adjustments in interstitial substance concentrations and downstream signaling cascade responsiveness. Rather, this type of mechanism might mechanically alter smooth muscle cell Ca^{2+} channel activation, which could provide an instantaneous link between contraction intensity and smooth muscle tone. Clifford et al. (2) have recently demonstrated that mechanical compression of resistance vessels causes an immediate vasodilation. However, these investigators did not examine the off-response of such vasoregulation. It remains to be examined whether altering the magnitude of mechanical vessel distortion results in directionally consistent rapid changes in vessel tone.

Symmetry of FBF_{relax} response to increase vs. decrease in contraction intensity. An even more intriguing observation in the current study was the remarkable symmetry of the decreases and increases in vascular tone with contraction intensity changes, and that this symmetry was the same in the slow and fast frequencies. To our knowledge, no data exist to describe the on- versus off-transient characteristics of vasodilatory mechanisms, much less rapid acting ones. Data from the current study speak to the nature of the rapid vasodilatory mechanisms determining cytosolic [Ca^{2+}] changes in smooth muscle, i.e., that the effectiveness in reducing (vasodilation) and restoring (removing dilation) cytosolic [Ca^{2+}] would appear to be similar.

This symmetry was identical for the first two contractures of a contraction intensity change in the slow and fast frequencies (Fig. 7). However, the ability to follow contraction intensity oscillations was absent in the very fast frequency trial (Fig. 5). This indicates that there is a frequency sensitivity of rapid vasoregulatory mechanism(s) such that, at high enough frequencies of contraction intensity change, they are unable to rapidly adjust vascular tone. However, below a threshold frequency of contraction intensity change, the immediate response dynamics are consistent and symmetrical.

In conclusion, this study has demonstrated that vascular tone can be rapidly and repeatedly regulated with changes in contraction intensity in human muscle. Furthermore, it has demonstrated a remarkable symmetry in the response dynamics to increases versus decreases in contraction intensity. We propose that the vasoregulatory mechanisms responsible are not likely to be substances released with muscle contraction but may instead be related to mechanical resistance vessel distortion with contraction. This hypothesis is testable, applying the recently developed technique of mechanical vessel distortion by Clifford et al. (2).

GRANTS

This study was supported by a Natural Sciences and Engineering Research Council (NSERC) Operating Grant (250367–02) and New Opportunities Infrastructure grants from Canada Foundation for Innovation and Ontario Innovation Trust (to M. E. Tschakovsky). N. R. Saunders was funded by an NSERC PGS-A scholarship award. K. E. Pyke was funded by an Ontario Graduate Student scholarship award.

REFERENCES


