Characterizing rapid-onset vasodilation to single muscle contractions in the human leg

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Credeur DP, Holwerda SW, Restaino RM, King PM, Crutcher KL, Laughlin MH, Padilla J, Fadel PJ. Characterizing rapid-onset vasodilation to single muscle contractions in the human leg. J Appl Physiol 118: 455–464, 2015. First published December 24, 2014; doi:10.1152/japplphysiol.00785.2014.—Rapid-onset vasodilation (ROV) following single muscle contractions has been examined in the forearm of humans, but has not yet been characterized in the leg. Given known vascular differences between the arm and leg, we sought to characterize ROV following single muscle contractions in the leg. Sixteen healthy men performed random ordered single contractions at 5, 10, 20, 40, and 60% of their maximum voluntary contraction (MVC) using isometric knee extension made with the leg above and below heart level, respectively. These were compared with single isometric contractions of the forearm (handgrip). Single thigh cuff compressions (300 mmHg) were utilized to estimate the mechanical contribution to leg ROV. Continuous blood flow was determined by duplex-Doppler ultrasonography and blood pressure via finger photoplethysmography (Finometer). Single isometric knee extensor contractions produced intensity-dependent increases in peak leg vascular conductance that were significantly greater than the forearm in both the above- and below-heart level positions (e.g., above heart level: leg 20% MVC, +138 ± 28% vs. arm 20% MVC, +89 ± 17%; P < 0.05). Thigh cuff compressions also produced a significant hyperemic response, but these were brief and smaller in magnitude compared with single isometric contractions in the leg. Collectively, these data demonstrate the presence of a rapid and robust vasodilation to single muscle contractions in the leg that is largely independent of mechanical factors, thus establishing the leg as a viable model to study ROV in humans.

vascular conductance; hyperemia; exercise onset; blood flow

AT THE ONSET of dynamic exercise, skeletal muscle blood flow responses are characterized by an initial rapid increase, followed by a slower rise to steady state (20, 39, 44). In an effort to focus on the rapidity of the blood flow responses to exercise, studies have been performed to assess the vasodilation following single 1-s isometric muscle contractions. This initial response following a single muscle contraction, termed rapid-onset vasodilation (ROV), is proportional to the intensity of contraction and peaks within five cardiac cycles post-contraction (8, 11, 30, 48, 51). ROV is considered to be an important initiating event to exercise hyperemia (8, 9). Previous investigations into ROV in humans have focused on the forearm as a model of study (2, 6, 12, 48). However, to our knowledge, no attention has been given to the lower extremities. The lower limbs exhibit large fluctuations in blood flow throughout the day due to periods of sitting, standing, and walking (32, 36, 47), and importantly, skeletal muscle contractions performed in this region are our primary means of locomotion. There are also known differences in resistance vessel function between the upper and lower extremities (31, 35), making extrapolation of findings from the arm to the leg difficult. Furthermore, arteries in the legs are at greater risk for developing atherosclerosis compared with other blood vessels in the body (26); thus, examining ROV in the leg may be an important model for elderly and patient studies in which impairments in the vasculature have previously been shown (2, 5, 14, 22, 25).

The mechanisms underlying ROV to single muscle contractions have not been fully elucidated; however, mechanical factors (10, 24, 50), as well as release of vasodilators from contracting skeletal muscle (e.g., K+ and the vascular endothelium (e.g., nitric oxide and prostaglandins) appear to play roles (7, 12, 18). Limb position also appears to have an important influence on ROV. Tschakovsky et al. (48) reported that initial increases (1st cardiac cycle post-contraction release) in blood flow and peak hyperemic responses to single muscle contractions in the forearm were significantly greater when the limb was lowered below heart level, as opposed to elevated above. The authors attributed this effect of limb position on ROV to be the consequence of a greater muscle pump contribution in the below-heart level limb position (50). Since these early ROV experiments in humans, subsequent examinations into ROV have focused on studying the limb above heart level to minimize the muscle pump contribution to the response, thus allowing for study of additional vasodilatory mechanisms contributing to ROV (2, 5–7, 12, 24).

Mechanical compression can also stimulate significant vasodilation (10, 24). Kirby et al. (24) showed in the human forearm that mechanical compression induced by a pneumatic forearm cuff resulted in significant vasodilation up to a maximum pressure of 300 mmHg. However, single muscle contractions were shown to elicit significantly greater and sustained vasodilatory responses compared with cuff compressions. Importantly, although vasodilation to single forearm contractions was intensity dependent, this was not the case for graded cuff pressures, suggesting that additional factors other than mechanical compression are stimulating vasodilation to a single forearm contraction (24).

With this background in mind, the purpose of this study was to characterize ROV following single muscle contractions...
performed in the leg. To do this, graded single muscle contractions using isometric knee extension were made with the leg above and below heart level. In addition, to better understand the mechanical component to ROV in the leg, hyperemic responses to a brief mechanical compression (pneumatic thigh cuff), independent of any muscle contraction, were measured. Finally, we compared ROV responses from the leg to that of the forearm. Given previous work demonstrating greater endothelial-dependent and -independent vasodilator responses in the forearm and increased alpha-adrenergic responsiveness in the leg vasculature (31, 35), we hypothesized that ROV responses would be greater in the forearm compared with the leg following a single isometric muscle contraction.

METHODS

Subjects

Sixteen young men participated in this study: 10 subjects for protocol 1 (characterizing ROV in the leg with comparisons to the arm) and 6 subjects for protocol 2 (characterizing the mechanical component to ROV in the leg). Because of prior work identifying sex differences and hormonal influences on limb blood flow at rest and during exercise (34, 45), we included men only in the study. All subjects were recruited from the University of Missouri Campus and surrounding Columbia, MO, area. All experimental procedures and measurements conformed to the Declaration of Helsinki and were approved by the University of Missouri Health Sciences Institutional Review Board. Subjects provided written informed consent prior to participating in the study. Subjects were healthy, nonsmokers, and recreationally active with no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications.

Experimental Procedures

Single isometric muscle contractions in the leg. Single isometric knee extension contractions were used to study leg ROV. To do this, a strap was secured around the subject’s right ankle and attached to a cable system equipped with a load cell force transducer (Transducer Techniques, Temecula, CA). The transducer voltage was outsourced to a computer interfaced with Powerlab acquisition (ADInstruments) and displayed on a monitor to provide a visual reference for subject viewing. For the above-heart leg position, subjects were positioned supine with both legs elevated and supported on a custom-built bench surrounding Columbia, MO, area. All experimental procedures and measurements conformed to the Declaration of Helsinki and were approved by the University of Missouri Health Sciences Institutional Review Board. Subjects provided written informed consent prior to participating in the study. Subjects were healthy, nonsmokers, and recreationally active with no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications.

Experimental Protocols

Single isometric muscle contractions in the forearm. To study arm ROV, single isometric forearm muscle contractions were performed using a handgrip dynamometer (Stoelting; Wood Dale, IL), equipped with voltage output to Powerlab for continuous acquisition and visual display, similar to knee extension studies. Subjects were positioned supine on an examination table with the right arm abducted ~80°. To examine the influence of arm position on ROV, the subject’s right arm was either elevated ~20 cm above heart level or lowered to the same degree using an adjustable bedside table and vacuum pads, similar to previous studies of forearm ROV (2, 5–7, 12, 24). Prior to experimental trials, subjects performed three to five handgrip MVCs, and the three highest values obtained were averaged and set as 100%.

Experimental Measurements

Cardiovascular measures. Heart rate (HR) was monitored continuously using lead-II surface ECG (Q710; Quinton, Bothell, WA). Continuous beat-to-beat blood pressure (BP) was measured via finger photoplethysmography obtained from the left middle finger (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Before Finometer recordings were obtained, return-to-flow calibrations were performed to ensure accurate BP values. In addition, BP was also taken on the right arm using automated sphygmomanometry (Welch Allyn; Skaneateles Falls, NY) before experimental trials were performed to further validate absolute BP values (13, 21).

Limb blood flow. Femoral and brachial artery diameter and blood velocity profiles were measured continuously using a high-resolution duplex-Doppler ultrasound system (Logiq P5; GE, Milwaukee, WI), as previously described by our laboratory (3, 13, 15, 33). For leg contractions, the common femoral artery of the right leg was scanned longitudinally, distal to the inguinal crease ~2–3 cm proximal from the bifurcation of the superficial and deep femoral artery branches. For forearm contractions, the right brachial artery was scanned longitudinally ~3–5 cm proximal from the antecubital fossa along the anterior-medial aspect of the upper arm. Continuous diameter and blood velocity measurements were obtained using a linear array transducer (10–12 MHz) in pulse-wave mode operating at a Doppler frequency of 5 MHz and insonated at an angle of 60° to the artery. Doppler-ultrasound measurements were performed with the velocity cursor set midway and sample volume adjusted to encompass dye lumen without extending beyond it. Probe placement was marked on the skin using a permanent marker to ensure consistent placement throughout experimental trials.

Familiarization Sessions

Prior to experimental visits, all subjects came to the laboratory and were familiarized with all experimental procedures and measurements. These sessions included screening of the common femoral and brachial artery to ensure the attainment of an adequate signal and to allow the subject to become comfortable with these measurements. In addition, MVCs were determined for the respective limbs and subjects were allowed to practice performing single (1-s) contractions at the relative exercise intensities used during experimental trials (5–60% MVC). To ensure a 1-s contraction was performed, subjects were coached using a metronome (Matrix, MR-500) set at cadence of 60 beeps/min. Essentially, the subject initiated a contraction during one beep and relaxed during the subsequent one. In addition, a virtual box was positioned on the monitor at the prescribed level of exercise to facilitate accuracy in achieving the target intensities for ROV trials. These helpful aids were also performed during all experimental trials.

Experimental Protocols

Prior to experimental study visits, all subjects were fasted for at least 3 h, refrained from caffeine intake for 12 h, and strenuous physical activity and alcohol consumption for 24 h. Subjects were studied in a dimly lit temperature-controlled room (21–22°C) and instrumented for continuous measures of BP, HR, and blood flow.

Protocol 1: characterizing ROV in the leg with comparisons to the arm. Leg and arm ROV experiments were performed on different days, which were randomized, and separated by at least 24 h. All experimental sessions were performed at the same time of day for repeat visits. The protocol for ROV experiments is summarized in a
schematic in Fig. 1. The order of the above- vs. below-heart level limb position was randomized. Following 20 min rest, a 2-min baseline recording of BP, HR, and blood flow was obtained prior to commencing ROV trials. For experimental trials, cardiovascular and blood flow data were collected continuously for 15 s prior to and for 45 s following release of each single (1 s) muscle contraction. Subjects performed three trials at 5, 10, 20, 40, and 60% of their MVC with 2 min of rest allotted between trials. Importantly, the intensity of exercise was also randomized, but trials were grouped by their respective intensity.

Protocol 2: characterizing the mechanical component to ROV in the leg. In a subset of subjects \((N = 6)\), hyperemic responses to mechanical compression, independent of muscular contraction, were induced by inflating a pneumatic cuff (Hokanson E20; Bellevue, WA) positioned around the right thigh halfway between the iliac crease and knee. Cardiovascular and blood flow measures were recorded continuously for 15 s prior to and for 45 s following release of a 300-mmHg occluding pressure performed in both the above- and below-heart level leg position. Two seconds were allotted between initiating cuff inflation and deflating to account for an approximate 1-s delay in the cuff achieving the desired pressure (24). A total of three cuff-inflation trials were performed with 2 min between trials. The rationale for using a 300-mmHg cuff inflation pressure stems from evidence suggesting that a 300-mmHg external pressure applied to a limb is comparable to intramuscular pressures seen during heavy leg exercise (1, 39, 43). Furthermore, in five subjects graded thigh-cuff inflation (100, 200, and 300 mmHg) were performed for both leg positions, and no significant differences were noted in the magnitude of change in blood flow between these pressures (data not shown). Therefore, a 300-mmHg extravascular pressure was used to maximize the mechanical component of ROV. These six subjects also performed single isometric leg contractions as described for protocol 1 to directly compare thigh cuff responses with ROV responses in the same subjects.

Data Analysis

All experimental measurements were acquired into a custom LabVIEW program interfaced with video output of the Doppler ultrasound machine as previously described in detail by our laboratory (13, 15, 16, 33). The ECG and BP signals were sampled at 1 kHz and embedded as data streams into an AVI file containing video images of the femoral and brachial arteries with corresponding blood velocity waveforms outsourced from the ultrasound at a sampling rate of 30 Hz. A custom-designed edge detection and wall tracking software (LabVIEW; National Instruments) was used to determine beat-by-beat arterial diameters and weighted mean blood velocity offline (13, 15, 16, 33). These data were processed using a second custom LabVIEW program, which generated synchronized beat-by-beat data of all recorded variables gated by the R-wave of the ECG. Artery diameter and mean blood velocity measurements were used to calculate blood flow as \(\pi \times (D/2)^2 \times V_{\text{mean}} \times 60\), where \(V_{\text{mean}}\) is mean blood velocity (cm/s) and \(D\) is arterial diameter (cm). Vascular conductance (VC) was calculated as \(V_{\text{mean}} = \text{blood flow/MAP} \text{ (ml·min}^{-1} \cdot \text{mmHg}^{-1}).\)

Changes in all variables were calculated from the average of 10 cardiac cycles of data preceding contraction (precontraction) or cuff inflation (pre-cuff inflation) and plotted over R-wave time starting with the first cardiac cycle uninterrupted by muscle contraction or cuff inflation. Changes in blood flow and VC in response to the three trials for each MVC intensity, and also cuff inflation, were averaged to provide an individual mean response for each subject, which were subsequently averaged for a group mean response. The initial response was determined as the first uninterrupted cardiac cycle following release of contraction or cuff inflation, and peak response was considered the maximal increase. Data are presented as absolute and percent changes from precontraction and/or pre-cuff inflation values. Total blood flow and VC responses were also calculated as the area under curve (AUC) using a trapezoidal model for up to 14 cardiac cycles following release of a single contraction. The rationale for calculating AUC for up to 14 cardiac cycles stems from our data showing that mean blood flow responses to single contractions returned to baseline in most cases by this point (see Fig. 2). To examine latency of responses, the time to peak change in VC was also determined.

To estimate the mechanical component to peak leg ROV for a single muscle contraction, the change in blood flow following the 300-mmHg cuff compression trials was compared with the peak responses for a low-intensity (20% MVC) and high-intensity (60% MVC) single muscle contraction. For this analysis, the peak increase in blood flow for the single muscle contraction was subtracted from baseline blood flow and compared with the change in blood flow from baseline observed during the same cardiac cycle following thigh cuff inflation. Because the increase in blood flow to thigh cuff inflation was brief, peaking within the first cardiac cycle, and returning towards baseline thereafter, this analysis approach avoided any overestimation of a mechanical contribution to the peak ROV response to single muscle contractions.

Statistical Analyses

Statistical analyses were performed using SigmaStat (Jandel Scientific Software; Chicago, IL). All data are presented as means ± SE. Paired \(t\)-tests were used to compare differences in cardiovascular variables between limb positions. Two-way repeated-measures ANOVAs were performed to examine intensity, positional, and limb differences in blood flow and VC following single muscle contractions. In addition, a two-way repeated-measures ANOVA was also performed to examine positional differences in leg blood flow responses following thigh cuff inflation, and finally, a one-way repeated-measures ANOVA was used to compare changes in blood flow following thigh cuff inflation and 20% and 60% MVC contractions. Bonferroni corrections were used for post hoc analyses when appropriate. Statistical significance was set at \(P \leq 0.05\).

RESULTS

Baseline Subject Characteristics

General subject characteristics are summarized in Table 1. Baseline cardiovascular variables between limb positions are
summarized in Table 2. Resting heart rate was significantly
greater in the below-heart leg position (P < 0.05), while
resting MAP was similar between all limb positions. Likewise,
no significant differences were noted for artery diameter, blood
velocity, blood flow, or vascular conductance at rest between
positions for the respective limb examined.

Protocol 1: Characterizing ROV in the Leg With
Comparisons to the Arm

Figure 2 shows the time course for blood flow responses
following single knee extensor contractions performed in the
above-heart (Fig. 2A) and below-heart (Fig. 2B) level posi-
tions. Following single isometric knee extension contractions,
there was an intensity-dependent increase in the initial (1st
cardiac cycle following contraction) and peak change in leg
VC for both the above- and below-heart leg positions (Fig. 3).
While the magnitude of the initial increase in leg VC was
similar between leg positions (Fig. 3A), the peak increases in
VC were greater in the below-heart level position, particularly
at the higher exercise intensities (20 – 60% MVC; Fig. 3B). The
time to the peak %change in leg VC was also greater in the
below-heart vs. above-heart level position but only at the lower
MVC intensities (5 and 10% MVC; Fig. 3C).

Greater blood flow and VC responses to single isometric
contractions were found in the leg compared with the forearm.

Figure 4 provides a beat-to-beat comparison of the hyperemic
responses to single isometric knee extensor and forearm con-
tractions in the above-heart level limb position. The largest
differences between limbs was found at the lower intensities of
contraction (5, 10, and 20% MVC). For the above-heart level
position, the limb differences in ROV were most notable for
initial and peak increases in VC (Table 3). Indeed, although
total AUC responses in VC also appeared greater in the leg,
statistical significance was only found at 10% MVC. For the
below-heart level position, the peak and total AUC responses
in VC were significantly greater in the leg during all exercise
intensities, further indicating greater ROV in the leg compared
with the forearm (Table 3). Interestingly, although initial in-
creases in VC following single isometric contractions were
greater in the leg compared with the forearm in the above-heart
position, initial VC responses were similar between limbs for
the below-heart level position (Table 3).

Protocol 2: Characterizing the Mechanical Contribution to
ROV in the Leg

The time course and magnitude of leg blood flow responses
following a low-intensity (20% MVC) and high-intensity (60%
MVC) single muscle contraction, along with the 300-mmHg
thigh cuff inflation are shown in Fig. 5. Single thigh cuff
compressions produced an initial rapid increase in blood flow
within the first cardiac cycle that quickly returned toward
baseline for the above-heart level leg position; however, the
vasodilatory response appeared more sustained in the below-
heart leg position (cardiac cycle P < 0.001; leg position P = 0.471;
interaction P < 0.001) with greater blood flow responses occurring from cardiac cycles 3 to 6 post-thigh cuff
inflation (P < 0.05 vs. above-heart level position). Neverthe-
less, in both leg positions it is clear that single muscle con-
tractions cause greater vasodilatation that continue for several
more cardiac cycles compared with thigh cuff inflation (Fig. 5).
Indeed, a comparison of the peak changes in blood flow to 20%
and 60% MVC single muscle contractions to responses follow-

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82 ± 3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>22 ± 1.3</td>
</tr>
<tr>
<td>Handgrip MVC, kg</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>*Knee extensor MVC, kg</td>
<td>37 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE, BMI, body mass index; MVC, maximal voluntary contraction. *Average of upright and supine MVCs.
ing thigh cuff inflation at the same cardiac cycle revealed a significantly greater increase in blood flow to contraction compared with thigh cuff inflation (e.g., above-heart level position: 60% MVC, 47 ± 30 ml/min, \(P < 0.05\)).

**DISCUSSION**

The primary purpose of this study was to characterize vasodilatory responses to single muscle contractions in the leg. We found that single isometric contractions in the leg produced a rapid, robust, and intensity-dependent increase in VC. This response was affected by limb position, such that increases in VC were greater when the leg was positioned below heart level. In addition, contrary to our hypothesis, we found that ROV in the leg was significantly greater than ROV in the forearm. Finally, mechanical cuff compressions induced a rapid but brief hyperemic response in the leg. Nonetheless, muscle contraction-induced vasodilation was greater than dilation produced by cuff compression. Collectively, these data demonstrate, for the first time, that single isometric muscle contractions in the leg stimulate ROV that is largely independent of mechanical factors.

**ROV Following Single Muscle Contractions in the Leg With Comparisons to the Forearm**

Previous work investigating ROV following single muscle contractions in humans have focused on the forearm as a model of study (2, 5–7, 12, 24, 48, 50). To our knowledge, no studies have specifically examined this phenomenon in the leg. Given known differences in vascular control between the upper and lower extremities (31, 35, 37, 41), as well as the greater propensity of arteries in the leg to develop atherosclerosis (26, 32), we were prompted to investigate ROV following single muscle contractions in the leg. We provide clear evidence for ROV in the leg of young healthy men.

Our results suggest that there are some similarities between ROV stimulated by single muscle contractions in the arm and leg. Indeed, as reported in the forearm (4, 5, 11, 12, 24, 48), we observed an intensity-dependent increase in leg VC following single isometric knee extension contractions, peaking at 5 s post-contraction release. VanTeeffelen and Segal (51) demonstrated in skeletal muscle arterioles that the magnitude of vasodilation following a single muscle contraction is proportional to the number of active motor units recruited to develop a given amount of force. We implemented graded intensities of single isometric knee extensor contractions in our study which would presumably increase the number of active motor units firing, thus resulting in the intensity-dependent vasodilatory response observed (48, 51).

Given previous reports of greater vasodilatory responses in the forearm and enhanced alpha-adrenergic vasoconstrictor responsiveness in the leg (31, 35), we hypothesized the leg would have smaller ROV responses. However, our findings indicate that peak changes in VC following single isometric muscle contractions in the leg were significantly greater than those in the forearm. This finding is consistent with previous reports indicating that the leg has a greater capacity to vasodilate in response to contractions compared with the forearm (31, 35, 37, 41).

**Table 2. Baseline cardiovascular variables**

<table>
<thead>
<tr>
<th></th>
<th>Leg</th>
<th>Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above heart</td>
<td>Below heart</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>54 ± 2</td>
<td>64 ± 3*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>93 ± 2</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>Diameter, cm</td>
<td>0.96 ± 0.03</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td>Mean blood velocity, cm/s</td>
<td>7.6 ± 1.1</td>
<td>6.2 ± 1</td>
</tr>
<tr>
<td>Blood flow, ml/min</td>
<td>327 ± 41</td>
<td>275 ± 28</td>
</tr>
<tr>
<td>Vascular conductance, ml·min⁻¹·mmHg⁻¹</td>
<td>3.48 ± 0.38</td>
<td>3.04 ± 0.32</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\(P < 0.05\) vs. above heart position; †\(P < 0.05\) vs. leg (respective limb position).

Fig. 3. Mean summary data showing the initial response (first cardiac cycle; A), the peak response (B), and the time to peak response (C) for leg vascular conductance from rest following single knee extensor contractions at varying intensities (5, 10, 20, 40, and 60% MVC). Data are depicted for above-heart level and below-heart level limb positions. Values are means ± SE. *\(P < 0.05\) vs. above-heart leg position.
contractions were greater in the leg compared with the forearm. The primary reason for the greater ROV responses in the leg is not clear. Indeed, the mechanisms underlying ROV are still incompletely understood. Factors released from contacting skeletal muscle, such as K⁺, can stimulate smooth muscle hyperpolarization and vasodilation (12, 18, 23). This concept was recently demonstrated in the human forearm by Crecelius et al. (12), where inhibition of K⁺-mediated hyperpolarization, as well as blockade of nitric oxide (NO⁻) and prostaglandin (PG) signaling pathways prior to a single muscle contraction nearly abolished the ROV response. However, the question of whether the mechanisms contributing to ROV following single muscle contractions are similar between arms and legs awaits investigation.

Because of the hydrostatic pressure differences between the arm and leg positions, it is plausible that the greater hydrostatic column with the leg set-up, particularly in the below-heart level position, contributed to the greater leg ROV responses observed. As described in METHODS, the positioning chosen for the leg ROV studies was necessitated to ensure force production was consistent for above- vs. below-heart leg comparisons. In terms of making limb comparisons, we relied more on the above-heart leg vs. above-heart arm positions to minimize the influence of hydrostatic force differences on ROV responses.

### Table 3. Comparison of cardiovascular responses to single muscle contractions between the leg and arm

<table>
<thead>
<tr>
<th>Limb</th>
<th>Intensity of Single Muscle Contraction</th>
<th>Factor P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MCV</td>
</tr>
<tr>
<td></td>
<td>Initial response, %VC</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>5%</td>
<td>32 ± 14</td>
</tr>
<tr>
<td>Arm</td>
<td>2 ± 4</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>Leg</td>
<td>10%</td>
<td>88 ± 22</td>
</tr>
<tr>
<td>Arm</td>
<td>16 ± 5</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Leg</td>
<td>20%</td>
<td>670 ± 188</td>
</tr>
<tr>
<td>Arm</td>
<td>133 ± 42</td>
<td>238 ± 54</td>
</tr>
<tr>
<td>Leg</td>
<td>Peak response, %VC</td>
<td>−0.3 ± 1.4</td>
</tr>
<tr>
<td>Arm</td>
<td>2.4 ± 1.1</td>
<td>0.2 ± 1.4</td>
</tr>
<tr>
<td>Leg</td>
<td>Total response, AUC VC</td>
<td>670 ± 188</td>
</tr>
<tr>
<td>Arm</td>
<td>133 ± 42</td>
<td>238 ± 54</td>
</tr>
<tr>
<td>Leg</td>
<td>HR, Δbeats/min at VC peak</td>
<td>−0.2 ± 0.6</td>
</tr>
<tr>
<td>Arm</td>
<td>1.9 ± 1.5</td>
<td>0.4 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. VC, vascular conductance; HR, heart rate; AUC, area under the curve. †Significantly different from arm response (P < 0.05).

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Figure 4. Mean summary data showing the time course for percent changes in blood flow (A) and vascular conductance (B) from rest following single isometric knee extensor (○) and forearm (●) contractions at varying intensities (5, 10, 20, 40, and 60% MVC) in the above-heart level position. The first 20 cardiac cycles following contraction are presented. Values are means ± SE. *P < 0.05, significant difference between limbs.

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In this regard, in the above-heart leg position, the leg has to actually overcome a much greater hydrostatic force to increase blood flow, yet leg ROV was still greater than the forearm. We believe this comparison further strengthens the finding that ROV was greater in the leg compared with the forearm and suggests hydrostatic differences do not fully explain the observed differences in ROV between the leg and forearm. A more plausible explanation for the greater ROV response in the leg compared with the forearm may be related to differences in skeletal muscle recruitment patterns between contractions. Despite marked differences in lean muscle mass between the thigh and forearm, the MVC was similar between isometric knee extension and forearm contractions, similar to prior studies (19, 42). Thus, for any given amount of force, the leg presumably has a greater recruitment of skeletal muscle. Given previous work demonstrating that contraction-induced dilation is directly related to the number of active motor units at a given tension (51), the greater ROV in the leg compared with the forearm might be due to between-limb differences in the number of motor units recruited during contractions. This may contribute to the greater disparity in ROV between the leg and forearm at the lower MVC intensities (Fig. 4); however, future studies will be needed to fully elucidate the observed limb differences in ROV.

**Above- vs. Below-Heart Limb Position Effects on ROV in the Leg**

Despite much debate (17, 27, 49, 50), the “muscle pump” phenomenon requires consideration when examining ROV. This concept implies that venous volume and pressure are reduced during compression of the vasculature via contraction, in turn widening the arterial-venous pressure gradient and subsequently increasing blood flow through the exercising region (17, 27, 36, 49, 50). Importantly, Tschakovsky et al. (48) reported that initial increases in blood flow (1st cardiac cycle) and peak hyperemic responses to single forearm muscle contractions were greater when the limb was positioned below heart level, possibly due to greater venous pooling in this position (17, 36, 47, 48). We found similar results in the forearm with the initial VC response being greater in the below-heart compared with above-heart position. However, we did not find a significant difference in the initial increase in VC following single contractions between the above- vs. below-heart leg positions. Although the reason for this positional limb difference is unclear, it is possible that for the leg, the majority of blood pooling occurred in the calf region (17), which minimized any muscle pump effect in the quadriceps when the subject was studied upright.

In the present study, we found that peak ROV responses were greater in the below-heart leg position compared with above-heart leg position. Similarly, previous evidence demonstrates that following passive limb movement, a model to study vascular function (46, 52), peak increases in leg blood flow were also greater in the upright posture compared with supine. The authors provided two possible explanations for this positional effect on leg blood flow responses. First, it may be a consequence of central hemodynamics due to a greater heart rate increase following passive leg movement in the upright posture. In addition, it was suggested that a greater hydrostatic column effect in the upright leg position may facilitate an increase in driving pressure, and subsequently movement of blood across the vasculature (46). In the present study, although heart rate increased during single isometric leg contractions, this occurred only at the highest exercise intensities (e.g., 40 and 60%). Since the leg ROV responses between limb positions were greater at the lower exercise intensities, in which no increases in heart rate were observed, our data suggest that cardiac changes likely are not contributing to the differences in ROV responses between positions. In fact, the heart rate responses were actually greater in the above-heart leg position, whereas ROV responses were greater in the below-heart leg position. Thus, as suggested by Trinity et al. (46), it
may be more likely that the greater vasodilatory response to single muscle contractions in the below-heart leg position in the present study were due to a greater hydrostatic column effect in this leg position. Additional studies will be needed to test this supposition.

Previous studies in rodents have demonstrated that activation of alpha-adrenergic receptors can oppose vasodilation to single skeletal muscle contractions (29). More recently, Casey and Joyner (5) demonstrated that acute increases in muscle sympathetic nerve activity, induced by lower-body negative pressure, can transiently decrease vasodilator responses to a single isometric muscle contraction in the human forearm. Therefore, it is possible that sympathetic nerve activity differences between the upright and supine posture may have influenced vascular responses to single isometric muscle contractions between leg positions. However, based on previous studies (40), muscle sympathetic nerve activity would be expected to be higher in the upright position (i.e., leg below heart), and that is when the largest ROV responses occurred. Thus it is unlikely that postural differences in sympathetic nerve activity contributed to the observed differences in ROV between leg positions.

Mechanical Contributions to ROV in the Leg

Mechanical deformation of the vasculature itself during muscular contraction can stimulate vasodilation. Clifford et al. (10) showed in rats that extravascular pressures applied to isolated skeletal muscle feed arteries resulted in significant vasodilation, with a temporal hyperemic profile similar to that seen following a brief muscle contraction. The authors attributed this response to be due to both endothelium-dependent and -independent mechanisms, as vasodilation to external pressure application still persisted following denudation of the endothelium (10). Active vasodilation to mechanical compression has also been demonstrated in vivo in humans (24, 50). Kirby et al. (24) indicated that a brief mechanical compression, via a pneumatic cuff applied to the forearm, induces rapid vasodilation in the forearm. In agreement, we now show that mechanical thigh-cuff compressions result in a rapid vasodilatory response in the leg, but in contrast to muscle contractions, this was short lived in duration and magnitude. Kirby et al. (24) also demonstrated that a nonlinear relationship exists between external cuff pressure application and the magnitude of peak vasodilation, such that cuff pressures between 25 and 100 mmHg were graded, but then a plateau occurred with no significant further dilation until 300-mmHg forearm cuff pressure was applied. More importantly, we found that the magnitude of change in blood flow to graded thigh-cuff inflations (100, 200, and 300 mmHg) was not different. Thus we believe our 300-mmHg thigh cuff represented a maximal compression-induced dilation. Given that compression-induced dilation was rapid and short lasting, we deduce that the contribution of mechanical factors to peak ROV in the leg is relatively small. Finally, we found that the total magnitude of mechanical-induced hyperemia was greater in the below-heart leg position. Tschakovsky et al. (48) reported a similar response in the forearm and attributed this to a “muscle pump”-mediated response. We reason that, similar to the observed positional difference in leg ROV following single contractions, the greater hydrostatic column (46) may also be facilitating a more sustained vasodilatory response to a brief cuff compression in the below-heart leg position (see Fig. 5B).

Perspectives

The present findings have important implications for investigating limb blood flow at the onset of exercise in humans. Previous research efforts have focused on the forearm as a model to examine ROV following single muscle contractions. For the first time we have established a model to assess ROV in the leg following single muscle contractions in healthy young individuals. Our data provide clear evidence regarding the temporality and magnitude of response for a given level of contraction, as well as positional influences on ROV following single leg contractions. Importantly, this model could be applied to other subject groups, such as the elderly and patient populations, where reductions in leg blood flow responses to steady-state exercise have previously been reported (22, 25, 28, 38). Whether impairments in leg ROV initiate the blunting of exercise hyperemia in these groups warrants investigation. Likewise, mechanistic studies of leg ROV are needed. Nevertheless, we now provide a foundation for the study of ROV in the vasculature of human skeletal muscle that is used for ambulation and also known to be more susceptible to atherosclerosis.

Summary

The findings of the present study demonstrate, for the first time, that single muscle contractions in the leg produce a rapid, robust, and intensity-dependent vasodilation in humans. In addition, we found that ROV in the leg was greater than ROV in the forearm. Importantly, although mechanical cuff compressions also induce a rapid hyperemic response in the leg, these were short in duration and lower in magnitude compared with single muscle contractions of the leg. Collectively, these data establish the presence of a rapid and robust vasodilation to single isometric muscle contractions in the leg that is largely independent of mechanical factors, thus establishing the leg as a viable model to study ROV in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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