Heterogeneous limb vascular responsiveness to shear stimuli during dynamic exercise in humans

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Wray, D. Walter, Abhimanyu Uberoi, Lesley Lawrenson, and Russell S. Richardson. Heterogeneous limb vascular responsiveness to shear stimuli during dynamic exercise in humans. J Appl Physiol 99: 81–86, 2005. First published February 17, 2005; doi:10.1152/japplphysiol.01285.2004.—Arm and leg vascular responsiveness to comparable shear stimuli during isolated dynamic exercise has not been assessed in humans. Consequently, six young cyclists performed incremental, intermittent handgrip exercise (arm) and knee-extensor exercise (leg) from 5 to 60% of maximal work rate (WR). Ultrasound Doppler measurements were taken in the brachial artery (BA), common femoral artery (CFA), and deep femoral artery (DFA) at rest and at each WR to assess diameter and shear rate changes. Exercise at 60% maximum WR increased shear rate to the same degree in the CFA (314.3 ± 33.3 s⁻¹) and BA (303.3 ± 26.3 s⁻¹), but was significantly higher in the DFA (712.6 ± 88.3 s⁻¹). Compared with rest, exercise at 60% maximum WR did not alter CFA vessel diameter, but increased BA diameter (0.42 ± 0.01 to 0.49 ± 0.01 cm) and DFA diameter (0.59 ± 0.05 to 0.64 ± 0.04 cm). These data from the DFA demonstrate for the first time a substantial improvement in vascular reactivity in a conduit vessel only slightly distal to the CFA. However, despite comparable dilation between the BA and DFA, the slope of the relationship between vessel diameter and shear rate was much greater in the arm (2.4 × 10⁻⁴ ± 4.6 × 10⁻⁵ cm/s) than in either the DFA (8.9 × 10⁻⁵ ± 1.5 × 10⁻⁵ cm/s) or CFA (2.1 × 10⁻⁵ ± 1.1 × 10⁻⁵ cm/s). Together, these findings reveal a substantial heterogeneity in vascular responsiveness in the leg during dynamic exercise but demonstrate that conduit vessel dilation for a given change in shear rate is, nonetheless, reduced in the leg compared with the arm.

Isolated dynamic arm or leg exercise provides wide-ranging hemodynamic and metabolic stimuli, a scenario with the potential for elucidating between-limb vascular differences. However, a comparison of arm vs. leg vascular responsiveness to similar shear stimuli during exercise has not been performed in humans, in part because blood flow measurements taken in different limbs are often difficult to compare directly. Using animal isolated vessel preparations, it has been estimated that vascular responsiveness to exercise-induced shear stimuli is minimal (21). In contrast, several studies in human conduit vessels have reported considerable vasodilation in the arm (BA) following reactive hyperemia (4, 52), controlled hyperemia (35), and rhythmic handgrip exercise (23, 46). In the leg, ultrasound Doppler measurements are typically taken in the CFA, 2–3 cm distal to the inguinal ligament and proximal to the CFA bifurcation, since imaging of this relatively large conduit vessel is possible even during high-intensity knee-extensor exercise (36, 40, 42). In contrast to the reactive BA, the majority of studies in the leg report little (24) or no (18, 32, 37, 53) discernible dilation of the CFA during exercise, despite large increases in leg blood flow and moderate increases in shear rate. Beyond the CFA bifurcation, the superficial femoral artery (SFA) continues distally as a conduit vessel, which perfuses the lower leg, whereas the deep femoral artery (DFA) supplies the large muscles of the upper leg. Ultrasound Doppler measurements in the DFA during exercise are technically challenging and are typically not performed in humans. However, considering that the DFA is in close proximity to a large locomotor muscle group and that DFA lumen diameter more closely resembles the BA (38), this vessel may represent a valuable additional site for between-limb comparisons.

While limb-specific hemodynamic responses during exercise are well recognized (1, 14, 43, 47), to our knowledge the fundamental comparison of arm and leg conduit vessel reactivity during isolated dynamic exercise has not been performed in humans. Consequently, the present study sought to test the general hypothesis that vascular reactivity in response to exercise is dependent on vessel lumen diameter, shear rate, and the limb in which the vessel lies. Specifically, we hypothesized that 1) dynamic forearm exercise would increase vessel diameter in the BA, but that CFA diameter would remain unchanged during single-leg knee-extensor exercise, despite similar shear rates in these two vessels; and 2) that the high blood flow and shear rate associated with dynamic knee-extensor exercise would elicit a change in diameter in the DFA similar to that seen in the BA during dynamic handgrip exercise.

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METHODS

Subjects and general procedures. Six young (20–38 yr), nonsmoking male cyclists participated in the present study. Informed consent was obtained according to the University of California San Diego Human Subjects Protection Program requirements, in accordance with the Declaration of Helsinki. As part of the initial subject screening, incremental knee-extensor (leg) and handgrip dynamometer (forearm) exercise tests were performed until failure to establish the maximal work rate (WR; WRmax) for each individual in each modality. Health histories and physical examinations were also completed. All studies were performed in a thermoneutral environment. Subjects reported to the laboratory in the fasted state and during data collection were in a semirecumbent position (~60° reclined) during leg measurements or supine during arm measurements, with the arm at heart level. Heart rate was recorded from a standard three-lead ECG (Logiq 7, GE Medical Systems, Milwaukee, WI).

Exercise modalities and exercise protocols. Single-leg knee-extensor exercise was performed, as described previously (2, 39, 40). The ergometer was adjusted so that contraction of the quadriceps muscles turned a flywheel producing an ~90°–170° arc of the lower leg. To provide progressive levels of resistance to the quadriceps muscles, tension was incremented by increasing friction on a belt surrounding the flywheel. Each subject completed a one-legged WRmax test lasting 8–10 min, with 10-W increases in WR each minute until the subject could no longer maintain a contraction frequency of 1 Hz.

One of the a priori study aims was to compare vascular responsiveness between the exercising arm and leg, which requires similar shear stimuli. As such, a single maximal voluntary contraction was established for each subject using a hydraulic handgrip dynamometer with an analog output (Rolyan Ability One, Germantown, WI), and this maximal voluntary contraction value was titrated so that intermittent handgrip exercise performed at 0.5 Hz produced shear rates in the BA that were similar to the CFA. Subjects were encouraged to perform two subjects to become familiar with the exercise equipment, ensuring that proper cadence and desired WR were achieved during the study day. For both arm and leg exercise, at each WR [5, 10, 20, 40, and 60% WRmax (WR0%max)], the subjects exercised for 2 min to ensure steady-state blood flow, with measurements taken during the 3rd min. In an effort to produce a comparable duty cycle and hyperemia for each muscle group, exercise cadence was 1 Hz for the leg (knee-extensor) exercise and 0.5 Hz for the forearm (handgrip) exercise. To avoid ordering effects, the sequence of arm and leg exercise bouts was randomized, with two subjects performing the exercise bouts on separate experimental days.

Ultrasound Doppler. The ultrasound system (Logiq 7, GE Medical Systems) was equipped with two linear array transducers operating at an imaging frequency of 7–8 and 10 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution existed. The CFA was insonated distal to the inguinal ligament, ~2–3 cm proximal to the bifurcation, and the DFA and SFA were insonated 4–5 cm distal to the CFA bifurcation. The BA was insonated approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle.

The blood velocity profile was obtained using the same transducers with a Doppler frequency of 4.0–5.0 MHz, operated in the high-pulsed repetition frequency mode (2–25 kHz) with a sample volume of 1.5–3.5 cm in depth. Care was taken to avoid aliasing using scale adjustments, especially during exercise. In duplex mode, real-time ultrasound imaging and pulse-wave velocity profile were viewed simultaneously. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of ≤60°. The sample volume was maximized according to vessel size and centered, verified by real-time ultrasound visualization of the vessel. Using artery diameter and mean blood velocity (Vmean), blood flow in the CFA and the BA was calculated as:

\[
\text{Blood flow (ml/min)} = V_{\text{mean}} \cdot \pi \cdot \left(\text{vessel diameter/2}\right)^2 \cdot 60
\]

To confirm the reproducibility of DFA diameter measurements, coefficient of variation values were calculated at rest and during incremental exercise (8 diameter measurements at each of the 5 exercise levels). However, movement artifact during exercise precluded accurate and repeatable velocity measurements in the DFA. This dichotomy may be better understood with an appreciation for the short period of time needed to provide a clear and analyzable image vs. the much longer, continuous time period needed for accurate Doppler measurements. Blood flow in the DFA was thus estimated mathematically from direct measurements in the CFA and SFA, calculated as:

\[
\text{Blood flow}_{\text{DFA}}(\text{ml/min}) = \text{blood flow}_{\text{CFA}} - \text{blood flow}_{\text{SFA}}
\]

During the 3rd min of each exercise level, ultrasound images (CFA, DFA, and BA) and Doppler velocity waveforms (CFA and BA only) were obtained. At all sample points, arterial diameter and angle-corrected, time- and space-averaged, and intensity-weighted Vmean values were calculated using commercially available software (Logiq 7, GE Medical Systems). Two ultrasound digital images and velocity spectra segments of 20 s each were recorded and saved to the GE Logiq 7 hard drive for offline image and waveform analysis.

Shear stress has been identified as a mechanism that stimulates the vascular endothelium and results in subsequent vasodilation (34). Blood viscosity was not measured, so shear rate was calculated using the equation (4):

\[
\text{Shear rate (s}^{-1}) = 4 \cdot \frac{\text{mean blood velocity (cm/s))}}{\text{diameter (cm)}}
\]

DFA shear rate was calculated using measured DFA diameter and estimated DFA blood velocity [blood velocity_{DFA} = blood flow_{DFA}/\left(\text{diameter}_{DFA}/2\right)^2 \cdot \pi \cdot 60].

Maximal \text{O}_2 consumption. On a separate day, subjects performed a graded maximal exercise test on a stationary bicycle (Quinton, Bothell, WA). After a 5-min warm-up, WR was increased each minute until the subject could no longer maintain a cadence >40 rpm. Oxygen consumption (\text{VO}_2) was measured with the TrueMax 2400 Metabolic Measurement System (ParvoMedics, Sandy, UT). Comparisons were made between resting and exercising vessel diameter and maximal \text{VO}_2 (\text{VO}_2_{max}) at WR0%max to evaluate the relationship among vessel size, vasomotor function, and aerobic capacity.

Data analysis and statistics. For each 20-s ultrasound Doppler segment, Vmean was averaged across the first and last 10 s of the recorded clip. Ultrasound vessel diameter measurements were evaluated during diastole and were in the relaxation phase of muscle contraction during the exercise protocol, with one diameter measurement used for each 10-s clip. Statistics were performed with the use of commercially available software (SPSS, Chicago, IL). Repeated-measures ANOVA was performed on the slope and Y-intercept of vessel diameter, change in diameter, and shear rate data across increasing WR. Regression analysis was performed to assess the relationship between \text{VO}_2_{max} and vessel diameter. All group data are expressed as means ± SE. Significance was established at P < 0.05.

RESULTS

Blood flow and diameter measurements. Good quality ultrasound images were attainable both at rest and during exercise in the BA, CFA, and DFA (Fig. 1). Compared with rest, exercise increased blood flow approximately sixfold in the arm (from 63 ± 14 to 389 ± 31 ml/min), with a concomitant ~15% increase in BA diameter (from 0.42 ± 0.01 to 0.49 ± 0.01 cm). As expected, blood flow was much greater in the CFA, increasing ~25-fold (from 157 ± 31 to 4,126 ± 354 ml/min) without
a significant increase in vessel caliber (from 1.06 ± 0.04 to 1.07 ± 0.04 cm). Similarly, SFA flow increased during exercise (from 31 ± 7 to 1.3777 ± 71 ml/min) without a change in vessel diameter. Calculated blood flow in the DFA also significantly increased during exercise (from 122 ± 24 to 2,796 ± 144 ml/min), accompanied by a ~9% increase in DFA diameter (from 0.59 ± 0.05 to 0.64 ± 0.04 cm). The repeatability of DFA diameter measurements was confirmed by coefficient of variation calculations at rest (1.4 ± 0%) and during exercise (1.6 ± 0.1, 1.8 ± 0.3, 1.6 ± 0.2, 1.1 ± 0.1, and 1.4 ± 0.1% at 5, 10, 20, 40, and 60% WRmax, respectively).

Shear rate values. Exercise at WR60%max increased shear rate in BA (from 57.4 ± 13.8 to 303.3 ± 23.7 s⁻¹) and the CFA (from 13.8 ± 2.6 to 314.28 ± 33.3 s⁻¹), while the calculated shear rate in the DFA was significantly higher than both CFA and BA values (from 39.5 ± 11.46 to 712.6 ± 88.3 s⁻¹, estimated) (Fig. 2).

Between-vessel comparisons of diameter and shear rate. From rest to WR60%max, the increase in vessel diameter was similar between the BA (0.07 ± 0.02 cm) and DFE (0.05 ± 0.01 cm), whereas the CFA was unchanged. However, the slope relating the change in lumen diameter to a given change in shear rate was greatest in the BA (2.4 × 10⁻⁴ ± 4.6 × 10⁻⁵ cm/s), which was significantly greater than the slope of both the DFA (8.9 × 10⁻⁵ ± 1.5 × 10⁻⁵ cm/s) and CFA (2.1 × 10⁻⁵ ± 1.1 × 10⁻⁵ cm/s) (Fig. 3).

Whole body VO₂ max, VO₂ max measured during cycle exercise ranged from 54–72 ml·min⁻¹·kg⁻¹. In the BA, both resting ($r^2 = 0.46$) and exercising ($r^2 = 0.91$) diameter (WR60%max) significantly correlated with absolute VO₂ max (Fig. 4), and the strength of this correlation was only slightly reduced when VO₂ was normalized for body weight. CFA and DFA diameters at rest and during exercise did not correlate with absolute or normalized VO₂ max ($r^2 \approx 0.01$).

**DISCUSSION**

The present study has identified a differential responsiveness between the vasculature of the arms and legs during dynamic exercise. Moderate-intensity dynamic exercise of an isolated limb increased shear rate to the same degree in two conduit vessels: one in the arm (BA) and one in the leg (CFA). The CFA diameter did not increase significantly in response to this increased shear rate, whereas the BA dilated significantly, indicating attenuated leg vascular responsiveness. In contrast, the DFA dilated during exercise, demonstrating for the first time a substantial improvement in vascular reactivity in a conduit vessel only slightly distal to the CFA. However, while the DFA and the BA dilated to a similar degree during exercise, the change in vessel diameter relative to the increase in shear rate remained significantly smaller in the DFA compared with the BA, reemphasizing the relative lack of vascular reactivity in the leg compared with the arm. The complex relationship between limb-specific vascular structure and function was further emphasized through the surprising correlation between VO₂ max and vessel diameter in the untrained arm, but not in the chronically trained leg. Together, these findings reveal a substantial heterogeneity in vascular responsiveness in the leg during dynamic exercise, but demonstrate that conduit vessel dilation for a given change in shear rate is, nonetheless, reduced in the leg compared with the arm.

**Arm and leg vascular differences.** One of the fundamental complications in between-limb comparison of vascular responsiveness is production of a comparable stimulus. Because conduit vessel dilation is largely governed by endothelial-mediated mechanisms (34), equivalent increases in shear across the endothelium are needed for an accurate comparison. In the present study, progressive intermittent handgrip exercise and knee-extensor exercise at WR60%max produced similar increases in shear rate in the BA and CFA (Fig. 2), despite obvious differences in muscle mass (5) and limb blood flow, allowing direct between-limb comparison of conduit vessel reactivity. At WR60%max, BA diameter increased 14%, whereas the CFA lumen diameter was unchanged, emphasizing the striking difference between the arm and leg vasculature to a similar shear stimulus. In contrast to the CFA, lumen diameter...
increased significantly in the DFA during WR60%max exercise. However, this greater DFA vascular reactivity must be viewed relative to the shear rate in the DFA vs. the BA for appropriate between-vessel comparison. Evaluation of vascular responsiveness using the change in vessel diameter for a given change in shear rate reveals that vasodilation in the DFA was indeed far less than in the BA (Fig. 3). Thus measurements in both the CFA and DFA suggest that the leg vasculature responds to increases in shear rate with a more moderate vasodilation than the arm.

Arterial vessel responsiveness during exercise. We have identified a significant dilation in a vessel (DFA) supplying the leg musculature during dynamic knee-extensor exercise (Fig. 3). To our knowledge, this is the first report of DFA diameter changes during exercise in humans. Recently, arterial dimensions were measured at rest in the CFA and DFA in relation to the tissue volume each vessel supplies, but diameter measurements were not reported during exercise (38). Others have evaluated arterial blood flow in the CFA, DFA, and also the SFA at rest and 2-min postexercise (Wingate test), but no exercising blood flow or diameter measurements were reported (20). Thus the present findings extend these observations by providing measurements of both CFA and DFA diameter during progressive, dynamic exercise (Fig. 3). These measurements reveal the interesting observation that, by moving only ~6 cm distal to the point of measurement in the unresponsive CFA, a dilation of ~10% in the DFA can be observed, most likely due, in part, to the increased shear in this smaller conduit vessel. However, even if the slope of shear rate vs. diameter for the CFA is extended to shear rates comparable with those calculated for the DFA, dilation in the CFA remains greatly attenuated, suggestive of inherently different responses within the same limb (Fig. 3).

The documented increase in vascular reactivity in the DFA compared with the CFA may be the result of measurements taken closer to the active muscle tissue, where the vasoactive milieu emanating from the exercising muscle may alter vascular smooth muscle tone via “ascending vasodilation” (7, 12, 44). The observed DFA dilation during exercise may also be partially attributed to myogenic responses (3, 28, 29) or to changes in neural control of the exercising vasculature (17, 50). Alternately, the DFA may simply have an augmented response to the elevated shear rate. While the present data do not address these mechanisms, our measurements provide the first functional, in vivo evidence of vascular reactivity in the DFA during exercise in humans, which contrasts starkly with the unresponsive CFA.

Mechanisms and implications of conduit vessel reactivity. In the present study, dynamic exercise provides a robust shear stimulus to the conduit vessels of the arm and leg. Exercise provokes increased limb blood flow via resistance vessel dilation (6, 25), which may ascend to the level of larger arteries (44). This decrease in vascular resistance and subsequent hyperemia also provokes flow-mediated dilation of conduit vessels (6, 9, 25, 42). The putative mechanisms governing exercise-induced vasodilation are incompletely understood, but are thought to include the shear-stress-induced release of nitric oxide, prostacyclins, and endothelin, and the direct effect of vasoactive metabolites emanating from the exercising muscle (6, 9, 12). These metabolic factors are also known to alter neural regulation of the exercising muscle vasculature (17, 50). Pressure-dependent myogenic responses (3, 29) and the mechanical influence of the muscle pump (27, 45, 51) may also contribute to the regulation of exercising muscle blood flow. Thus, with this experimental model, it is clear that shear rate is
far from a mutually exclusive mechanism, but instead acts in combination with many other recognized factors, which collectively govern the limb-specific control of muscle blood flow (i.e., in the BA, CFA, and DFA) during exercise.

In a recent study, blood velocity and arm vessel diameter were highly correlated, whether velocity was increased by cuff occlusion ischemia, handgrip exercise, or local muscle heating, with the conclusion that vascular responsiveness can be measured as the slope of the velocity-diameter relationship, regardless of the stimulus that elevated blood velocity (48). However, findings from the present study propose a more complex relationship between vessel diameter and blood velocity in the exercising muscle. If the current data were viewed exclusively in terms of the velocity-diameter relationship, we would expect that the DFA should vasodilate much more than the BA, as calculated DFA \( V_{\text{mean}} \) was more than four times that of the BA during exercise at \( \text{WR}_{60\%\text{max}} \). In fact, both the CFA and DFA were much less reactive than the arm, despite higher blood velocities. Consequently, the present findings extend these observations in the arm to suggest that the degree of vascular responsiveness to blood velocity (shear rate) is also heavily dependent on the limb in which the vessel resides.

The observed limb-specific vascular responsiveness may have teleological implications during whole body maximal exercise, when leg vascular conductance increases dramatically. While recognizing that vascular conductance in the leg is largely dictated by the “downstream” resistance vessels, it is inviting to speculate that a decreased vascular responsiveness documented here in the large vessels of the leg might infer an overall diminution in leg vascular responsiveness. As noted by others (17, 22), reduced vasomotion in an exercising limb may serve as an additional mechanism by which adequate tissue perfusion and systemic arterial blood pressure are simultaneously maintained. Future studies are needed to further evaluate the potential importance of differential arm and leg vascular responsiveness to the maintenance of systemic cardiovascular homeostasis.

Conduit vessels and metabolic capacity. In the present study, all subjects were competitive cyclists and were considered to be chronically leg trained. Based on previous studies showing vascular remodeling with exercise training (11, 19, 30), it is probable that this has taken place to some degree in these subjects. In this context, the lack of a correlation between \( \text{VO}_{2\text{max}} \) and leg vessel diameter (both CFA and DFA) may at first appear surprising (Fig. 4). However, in the chronically trained limb, many other training-induced structural and metabolic adaptations have likely occurred within the skeletal muscle that may overshadow the impact of conduit vessel size on \( \text{VO}_{2\text{max}} \). Conversely, in these cyclists, a chronic training effect of the arm muscle itself and brachial vascular remodeling are not likely (11), yet the arm vasculature is exposed to the systemic hemodynamic effects that accompany endurance training. Thus the potential training-induced improvement in arm vascular function (10) devoid of vascular remodeling and muscular adaptations may explain why the arm conduit vessel size is more closely correlated to \( \text{VO}_{2\text{max}} \) in these subjects. In agreement with this concept, the relationship between brachial diameter and \( \text{VO}_{2\text{max}} \) was improved during exercise compared with rest (Fig. 4). Thus this comparison appears to afford us the opportunity to contrast the role of structure alone (resting correlation) and the combined influence of structure and function (exercise correlation) on the relationship between \( \text{O}_2 \) delivery and \( \text{VO}_{2\text{max}} \).

Potential study limitations. Ultrasound Doppler measurements were possible in the CFA and SFA, both at rest and during exercise. While clear ultrasound images of the DFA remained possible during exercise, the present study relied on an estimation of blood velocity in the DFA due to vessel movement artifact. However, ultrasound Doppler measurements made at rest in the DFA confirm that calculated blood flow through the DFA was arithmetically correct when estimated from CFA blood flow minus SFA blood flow. While it is difficult to directly compare arms and legs with any exercise modality, the approach of establishing a maximal intensity for both arm and leg exercise and then comparing submaximal intensities with similar shear rates allowed a reasonable point of comparison. We also recognize that orthostatic stress differed somewhat between arm (supine) and leg (seated) exercise, which was due to limitations in the exercise equipment. While the present study design sought to identify the impact of shear stimuli on vascular responsiveness, we cannot exclude the additional influence of the myogenic response, ascending vasodilation, and altered neural control of the vasculature on vascular reactivity. Finally, it is noteworthy that all subjects in the present study were cyclists with potential training-induced adaptations.

Summary. We have identified a differential responsiveness between the vasculature of the arms and legs during dynamic exercise that is complicated by the site of measurement. During exercise up to \( \text{WR}_{60\%\text{max}} \), CFA diameter did not change significantly, despite large increases in vascular shear rate, whereas significant dilation was observed in the slightly distal DFA. However, the change in vessel diameter relative to the increase in shear rate was still much less in the DFA compared with the BA. These data reveal a substantial heterogeneity of vascular reactivity within the conduit vessels of the leg, but that responsiveness to shear stimuli is, nonetheless, diminished compared with such vessels in the arm.

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