Brachial Artery Blood Flow Responses to Different Modalities of Lower Limb Exercise

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ABSTRACT


Introduction/Purpose: Cycling is associated with a reproducible systolic anterograde and diastolic retrograde flow pattern in the brachial artery (BA) of the inactive upper limb, which results in endothelial nitric oxide (NO) release. The purpose of this study was to examine the impact of different types and intensities of lower limb exercise on the BA flow pattern. Methods: We examined BA blood flow and shear rate patterns during cycling, leg kicking, and walking exercise in 12 young subjects (24 ± 3 yr). BA diameter, blood flow, and shear rate were assessed at baseline (1 min) and at three incremental intensity levels of cycling (60, 80, and 120 W), bilateral leg kicking (5, 7.5, and 10 kg), and walking (3, 4, and 5 km h⁻¹), performed for 3 min each. Edge detection and wall tracking of high-resolution B-mode arterial ultrasound images, combined with synchronized Doppler waveform envelope analysis, were used to calculate conduit artery diameter and anterograde/retrograde blood flow and shear rate continuously across the cardiac cycle. Results: BA mean blood flow and shear rate increased significantly throughout each exercise protocol (P < 0.001), and BA anterograde blood flow and shear rate showed comparable increases throughout each protocol (P < 0.001). Retrograde blood flow and shear rate, however, demonstrated a significant increase during cycling and walking (P < 0.001) but not during leg kicking. Conclusion: Rhythmic lower limb exercise (cycling and walking) results in an increase in BA systolic anterograde blood flow and shear rate, directly followed by a large retrograde flow and shear rate. This typical pattern, previously linked with endothelial NO release, is not present during a different type of exercise such as leg kicking. Key Words: SHEAR RATE, BLOOD FLOW PATTERN, HIGH-RESOLUTION ULTRASOUND, DOPPLER

Exercise training is a well-established and potent physiological stimulus to improve cardiovascular health and decrease the risk of cardiovascular diseases (11,21). One proposed mechanism that explains this relationship is the improvement in endothelial function induced by exercise training (12). Several putative mechanisms and physiological stimuli are linked with exercise-induced adaptations in endothelial function in active and nonactive regions (8,19), including an increase in endothelial shear stress (15).

During lower limb cycle exercise, blood flow through the brachial artery of the resting upper limbs undergoes a typical change in pattern; that is, a small increase of anterograde flow occurs during systole, followed by substantial increases of retrograde diastolic flow (6,7). In a previous series of experiments, we demonstrated that this oscillatory blood flow pattern in the brachial artery during leg exercise leads to endothelial release of the vasodilator nitric oxide (NO) (9). The importance of shear pattern changes was demonstrated by comparing these data with handgrip exercise, which results in a different shear pattern (primarily anterograde flow), and was not linked with substantive endothelial release of NO (9). These findings suggest that different types of shear rate pattern have a different impact on endothelial release of NO and they highlight the potential relevance of the pattern of shear rate to the magnitude of vascular adaptation that may occur with training.

On the basis of these findings, it is hypothesized that lower limb exercise induces improvements in upper limb endothelial function because of the typical change in flow patterns seen in the nonactive upper limbs (8,19). Different shear rate patterns observed during distinct types of exercise may have a different effect on adaptations. This hypothesis is supported by the finding that brachial artery endothelial function was found to improve after cycling and walking exercise training (12,14,16). In marked contrast, improvements in brachial artery endothelial function were...
not reported after a high-intensity resistance training, predominantly involving leg kicking exercise (3,16). The underlying mechanism for these distinct findings may relate to different brachial artery blood flow patterns observed during the different types of lower limb exercise. To provide insight into this hypothesis, the aim of the present study was to examine the impact of different types of lower limb exercise on brachial artery blood flow and shear rate pattern in healthy subjects. To this end, we examined brachial artery blood velocity and diameter continuously across the cardiac cycle during three incremental levels of cycling, leg kicking, and walking. We hypothesized that the changes observed in retrograde and anterograde shear rate in the nonactive brachial artery during the cyclic activities (e.g., cycling and walking) would not be evident in response to leg kicking exercise.

METHODS

Subjects

Twelve healthy recreationally active volunteers (6 men and 6 women, 23.9 ± 3.3 yr, 23.3 ± 1.9 kg·m⁻²) were recruited from the community. No subject reported having been diagnosed with cardiovascular disease, diabetes, insulin resistance, or cardiovascular risk factors such as hypercholesterolemia or hypertension. Subjects who smoked or were on medications of any type were excluded. Women were tested in the first week of the menstrual cycle to minimize any possible hormonal influence. The study procedures were approved by the Ethics Committee of Liverpool John Moores University and adhered to the Declaration of Helsinki; all subjects gave prior written consent.

Experimental Design

Participants reported to the laboratory after a ≥6-h fast, a ≥8-h abstinence from caffeine, and at least 24 h after strenuous physical activity. Three different exercise types were performed in a randomized order, i.e., cycling, leg kicking, and walking. These exercise types were chosen on the basis of their frequent use during exercise training studies. Each exercise session started with a resting period of at least 10 min, followed by 1 min recording of baseline. Thereafter, subjects performed three incremental intensity intervals of 3 min for each exercise type. Brachial artery diameter and blood velocity were recorded continuously throughout each exercise session.

Experimental Procedures

Brachial artery diameter and blood flow during lower limb exercise. To examine brachial artery diameter, Doppler velocity, blood flow, and shear rate, a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (t3000; Terason, Burlington, MA) was used to image the brachial artery in the distal third of the upper arm. Ultrasound parameters were set to optimize longitudinal, B-mode images of the lumen/arterial wall interface. Continuous Doppler velocity assessment was also obtained using the t3000 (Terason) and was collected using the lowest possible insonation angle (always <60°), which did not vary during each study. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap Pro 300V2; GE Healthcare, Tampa, FL) on the contralateral arm.

Cycling. After a resting period of at least 10 min on a recumbent cycle ergometer (Kettler XS1; Kettler Ltd., Worcestershire, United Kingdom), brachial artery baseline diameter, blood flow, and shear rate were recorded for 1 min. In addition, baseline heart rate and mean arterial pressure were recorded during this minute. Each subject performed three incremental cycle intensities (i.e., 60, 80, and 120 W, according to the protocol used in a previous study [9]). Subjects were instructed to keep a comfortable pedaling rate of approximately 60 rpm. The right arm was positioned at the level of the heart and was supported by a frame and foam to allow a stable position for brachial artery recordings. Each exercise bout was performed for 3 min. During the last minute of each exercise bout, arterial blood pressure was recorded on the contralateral arm.

Bilateral leg kicking. After a resting period of at least 10 min on the knee extensor machine (Leg Extension; Technogym, Gambettola, Italy) brachial artery baseline diameter, blood flow, and shear rate were recorded for 1 min. In addition, baseline heart rate and mean arterial pressure were recorded during this minute. Each subject performed three incremental bilateral knee kicking intensities at 5, 7.5, and 10 kg. On the basis of pilot studies, we observed that these workloads increased heart rate and induced local muscle fatigue yet allowed the assessment of echo Doppler recordings at acceptable quality levels for optimal analysis. Subjects were positioned on the knee extensor machine to allow a range of motion of the knee of 0°–90°, with the bar fixed immediately above the ankle joints. The arm was positioned at the level of the heart and was supported by a frame and foam to allow a stable position for brachial artery recordings. Using a metronome, subjects were instructed to extend and flex both legs simultaneously across a 3-s cycle. Each exercise bout was performed for 3 min. During the last minute of each exercise bout, arterial blood pressure was recorded on the contralateral arm.

Walking. Brachial artery baseline diameter, blood flow, and shear rate were recorded during the last minute of a 10-min resting period on a motorized-driven treadmill (Woodway, Auf-Schrauben, Germany). In addition, baseline heart rate and mean arterial pressure were recorded during this minute. While standing or walking, the right arm rested on a wooden support attached to the treadmill. This support was covered with foam to ensure a comfortable position for the subjects and to allow a normal gait pattern. Each subject walked at three incremental speeds (i.e., 3, 4, and 5 km·h⁻¹). On the basis of pilot studies, these walking speeds increased heart rate without affecting the quality of the echo Doppler recordings. Each exercise bout
was performed for 3 min. Subjects were instructed to walk at their voluntary chosen cadence. During the last minute of each exercise bout, arterial blood pressure was recorded.

**Brachial Artery Diameter and Blood Flow**

Posttest analysis of the brachial artery diameter and velocity was performed using custom-designed edge-detection and wall-tracking software that is largely independent of investigator bias (23). Briefly, the echo Doppler signal was real-time encoded and stored as a digital file. Subsequent software analysis of these data was performed at 30 Hz using an icon-based graphical programming language and toolkit (LabVIEW™ 6.02; National Instruments, Austin, TX). The initial phase of image analysis involved the identification of regions of interest (ROI) on the first frame of every individual study. These ROI allowed automated calibration for diameters on the B-mode image and velocities on the Doppler strip. An ROI was then drawn around the optimal area of the B-mode image, and within this ROI, a pixel–density algorithm automatically identified the angle-corrected near and far-wall e-lines for every pixel column within the ROI. The algorithm begins by dividing the ROI into an upper half, containing the near-wall lumen-intima interface, and a lower half containing the far-wall interfaces. The near-wall intimal edge is identified by a Rake routine that scans from the bottom to the top of the upper half of the ROI. The position of the edge is established by determining the point where the pixel intensity changes most rapidly. Typical B-mode ROI therefore contained approximately 200–300 diameter measures per frame, the average of which was calculated and stored. This process occurred at 30 frames per second.

A final ROI was drawn around the Doppler waveform and automatically detected the peak of the waveform. The mean diameter measure derived from within the B-mode ROI (above) was synchronized with the velocity measure derived from the Doppler ROI at 30 Hz. Ultimately, from these synchronized diameter and velocity data, blood flow (the product of cross-sectional area (CSA) and Doppler velocity (v)) and shear rate (four times velocity divided by diameter) were calculated at 30 Hz. All data were written to file and were retrieved for analysis in a custom-designed analysis package. We have shown that reproducibility of diameter measurements using this semiautomated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver CV of 6.7%. Furthermore, our method of blood flow assessment is closely correlated with actual flow through a “phantom” arterial flow system (10).

**Data Analysis**

Baseline diameter, flow, and shear rate were calculated as the mean of data acquired across the 1 min preceding exercise. During lower limb exercise, diameter, blood flow, and shear rate were calculated as the mean of data acquired across an interval of at least 10 s during the last minute of the exercise level. Calculation of brachial artery diameter, blood flow, and shear rate during exercise was, therefore, similar as reported in a previous study of our group (9).

**Statistics**

Statistical analyses were performed using SPSS 14.0 (SPSS, Chicago, IL) software. All data are reported as mean (SD), and statistical significance was assumed at $P \leq 0.05$. Repeated-measures ANOVA and post hoc paired t-tests (with Tukey’s correction for multiple comparisons) were performed.

### TABLE 1. Baseline resting blood pressure, heart rate, and brachial artery diameter before and during three incremental levels of cycling (60, 80, and 120 W, respectively), leg kicking (5, 7.5, and 10 kg, respectively), and walking (3, 4, and 5 km h⁻¹, respectively) in 12 healthy young subjects.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td>112 ± 15</td>
<td>137 ± 14*</td>
<td>143 ± 21*</td>
<td>157 ± 23*</td>
<td>&lt;0.001</td>
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<tr>
<td>Cycling (n = 10)</td>
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<tr>
<td>Kicking (n = 12)</td>
<td>114 ± 16</td>
<td>127 ± 18</td>
<td>125 ± 14</td>
<td>131 ± 10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td>65 ± 15</td>
<td>72 ± 11</td>
<td>70 ± 10</td>
<td>76 ± 12</td>
<td>&lt;0.05</td>
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<tr>
<td>Cycling (n = 10)</td>
<td></td>
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<tr>
<td>Kicking (n = 12)</td>
<td>72 ± 10</td>
<td>80 ± 7</td>
<td>66 ± 8</td>
<td>69 ± 11</td>
<td>NS</td>
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<tr>
<td><strong>Mean arterial blood pressure (mm Hg)</strong></td>
<td>81 ± 11</td>
<td>93 ± 10*</td>
<td>94 ± 13*</td>
<td>103 ± 12*</td>
<td>&lt;0.001</td>
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<td>Cycling (n = 10)</td>
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<tr>
<td>Kicking (n = 12)</td>
<td>86 ± 12</td>
<td>95 ± 8</td>
<td>85 ± 10</td>
<td>90 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>85 ± 5</td>
<td>100 ± 10*</td>
<td>108 ± 11*</td>
<td>113 ± 14*</td>
<td>&lt;0.001</td>
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<tr>
<td>Cycling (n = 12)</td>
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<tr>
<td>Kicking (n = 12)</td>
<td>77 ± 15</td>
<td>82 ± 14*</td>
<td>87 ± 14*</td>
<td>100 ± 17*</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Brachial artery diameter (mm)</strong></td>
<td>3.7 ± 0.7</td>
<td>3.6 ± 0.6</td>
<td>3.6 ± 0.7</td>
<td>3.6 ± 0.7</td>
<td>NS</td>
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<tr>
<td>Cycling (n = 12)</td>
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<tr>
<td>Kicking (n = 12)</td>
<td>3.5 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>3.6 ± 0.6</td>
<td>3.6 ± 0.4</td>
<td>NS</td>
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<tr>
<td><strong>Posttest analysis</strong></td>
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</table>
| Differences in blood pressure responses among exercise types were not performed. Data are presented as mean ± SD. * Significantly different from baseline, $P < 0.05$. † Post hoc significantly different from baseline, $P < 0.05$. NS = not significant.
used to assess the impact of lower limb exercise on brachial artery blood flow and shear rate patterns. In addition, a two-way repeated-measures ANOVA was used to examine whether the impact of lower limb exercise on brachial artery blood flow and shear rate pattern differed among the three exercise modalities.

RESULTS

Baseline resting blood pressure and brachial artery diameter, blood flow, and shear rate were not significantly different among the three exercise sessions, although heart rate was slightly higher before walking (Table 1). During exercise, heart rate, systolic blood pressure, and mean blood pressure increased significantly (all \( P < 0.05 \), ANOVA). The increase in heart rate during cycling was significantly larger than during leg kicking and walking (two-way ANOVA, post hoc; \( P < 0.05 \)). Diastolic blood pressure increased during cycling and kicking exercise but not during walking (Table 1). Brachial artery diameter did not change in any of the three exercise types (Table 1).

Brachial Artery Blood Flow

In Figure 1, a typical recording of one subject is depicted during cycling, leg kicking, and walking. Brachial artery mean blood flow increased significantly throughout each exercise protocol, with leg kicking demonstrating the largest increase in brachial artery blood flow (Fig. 2A). Mean blood flow during cycling and walking showed only a minimal increase. Brachial artery anterograde blood flow demonstrated a gradual increase throughout each protocol. The increase in brachial artery anterograde blood flow was comparable among the three different exercise types (Fig. 2B).

FIGURE 1—Screen captures of B-mode ultrasound images representing brachial artery diameter and velocity at rest (A) and at the first intensity level during cycling (B: 60 W), walking (C: 3 km h\(^{-1}\)), and leg kicking (D: 5 kg) for one subject. Diameter and cross-sectional area of the artery are calculated for each B-mode frame (at 25–30 Hz) using a Rake algorithm that detects the edges of the near and far walls within the selected arterial region of interest (yellow box). Then, 200–400 individual measurements are taken and angle-corrected along these edges, with the median being calculated as the final single composite diameter for that frame. Velocity is calculated via gray scale filtering using an automatic threshold algorithm with subsequent binary interrogation of each pixel column to detect the waveform envelope. Note the substantial change in negative (retrograde) velocity during cycle exercise (B) and during walking (C), although this is not evident during leg kicking exercise (D). Anterograde velocity, however, increases in all three different exercise modalities (B–D) compared with resting conditions (A). *Post hoc significantly different from other baseline values at \( P < 0.05 \).
Retrograde blood flow, however, demonstrated a significant increase throughout the incremental exercise bouts of cycling and walking, whereas retrograde blood flow did not change during leg kicking (Fig. 2B). The increase in retrograde blood flow during cycling was slightly, but significantly, larger than that observed during walking ($P = 0.03$, two-way ANOVA).

**Shear Rate**

During leg kicking and walking, mean shear rate demonstrated a gradual increase at each intensity level (Fig. 3A). Cycling exercise induced an initial decrease in shear rate, immediately followed by a modest increase at higher intensity levels (Fig. 3A). Mean shear rates during leg kicking, at each intensity level, were markedly higher than during cycling and walking. Brachial artery anterograde shear rate increased similarly throughout each exercise protocol, at each intensity level (Fig. 3B). Retrograde shear rate showed a significant increase throughout the cycling and walking protocols, which was not different between both exercise modalities. In contrast, retrograde shear rate did not change during leg kicking (Fig. 3B).

**DISCUSSION**

Examining brachial artery flow and shear rate patterns in response to different modalities of lower limb exercise, we observed that rhythmic lower limb exercise modalities (cycling and walking) result in a typical shear rate pattern change in the nonactive brachial artery of healthy young men and women. This involves an increase in systolic anterograde flow and shear, immediately followed by a large increase in diastolic retrograde flow and shear. Although our findings during leg cycling essentially confirm previous reports (6,7), the observations during walking exercise provide new insights in several respects. Remarkably, walking induces the same change in oscillatory shear pattern in the brachial artery during exercise as that previously observed during leg cycling exercise, a pattern which is linked with endothelial NO release. Because walking exercise training has beneficial effects on brachial artery endothelial function (1,23), our observed changes in upper limb shear may contribute to these adaptations. Moreover, the magnitude of these changes in shear during walking was observed at much lower heart rate intensities than during cycling exercise. Conversely, leg kicking, a more experimental form of
exercise, is primarily linked with a systolic blood pressure–driven change in anterograde shear rate, without a change in the retrograde component of the shear rate pattern. Previous studies involving these forms of lower limb exercise training have often not been associated with enhanced endothelial function (3,16). These observations raise the possibility that the differences in brachial artery shear rate patterns to cycling, walking, and leg kicking may contribute to the distinct exercise training–induced vascular adaptations evident between these different exercise modalities.

Remarkably, the largest effect of exercise on mean blood flow was observed during leg kicking. This large impact of leg kicking on blood flow cannot be explained by the intensity level because heart rate demonstrated a smaller increase during leg kicking than during cycling or walking (Table 1). This large increase in mean blood flow can partly be explained by the increase in systolic blood pressure, leading to an increased driving pressure. However, during cycling exercise, a comparable increase in systolic blood pressure is observed, whereas mean blood flow and shear rate levels are somewhat lower than during leg kicking. The exercise-induced changes in blood pressure, therefore, cannot fully explain our observations relating to anterograde flow and shear. Clearly, looking at the mean blood flow only does not reveal the underlying pattern of change. Indeed, the pattern of shear, consisting of the systolic anterograde and diastolic retrograde shear rate, markedly differs among the three exercise modalities. The increase in anterograde shear rate is in fact comparable among walking, cycling, and leg kicking. In contrast, retrograde shear rate does not change during leg kicking, although it increases markedly during the rhythmic activities of walking and cycling. As mean shear rate is the product of anterograde and retrograde shear rate, the larger mean shear rate during leg kicking is primarily explained by the minimal retrograde shear. Such findings have previously been described for handgrip versus cycle exercise (7,9). Our observations, therefore, reinforce previous findings that emphasize the importance of examining the complete shear pattern, rather than solely looking at average values, even where the latter may be calculated over one or more heartbeats.

Rhythmic exercise, such as walking, have deep roots in the evolution of humans (2). This is supported by the fact that these types of exercise can be continued for several hours (approximately 14 h [14,17]), whereas an average intensity level during such exercise is maintained at 70%–80% of the individual’s heart rate (4). In contrast, a bout of high-intensity exercise training, such as leg kicking, is
mostly continued maximally for 10 min (3), although heart rate does not increase as observed during aerobic exercise training. Not surprisingly, and in line with our hypothesis, walking and cycling, both representing cyclic and rhythmic exercise modalities, demonstrate comparable blood flow and shear rate patterns in the nonactive brachial artery. Although the change in retrograde blood flow is slightly higher during cycling than during walking exercise, this might be explained by individual differences in intensity level. For example, taking differences in heart rate in account, an accepted method to correct for individual differences in intensity, walking and cycling demonstrate similar changes in brachial artery anterograde and retrograde blood flow and shear rate at a given heart rate.

In a series of studies, we provided experimental evidence that the cycling-induced change in shear rate in the nonactive brachial artery is responsible for endothelial release of NO (9). Interestingly, walking exercise induces a similar change in brachial artery shear rate pattern as present during cycling exercise. Although cycling (12,14,16) and walking exercise training (1,22) have been related to the improvement in NO-dependent endothelial function, studies with local blockade of NO during walking will be necessary to gain better insight into the role for NO explaining the cardioprotective effects of walking, notwithstanding the likely technical difficulties. Recent studies of endothelial cells in culture suggest that some oscillatory flow patterns produce proatherogenic gene expression, decrease NO bioavailability, and promote endothelial dysfunction (13). Therefore, further basic and applied studies are required to gain better insight into possible mechanisms underlying the cardioprotective effects of exercise. In addition to the shear rate pattern, the duration of a period of increased shear rate might also be of relevance to explain the cardioprotective effect of exercise.

Because we have not used surface electromyography, a potential limitation of this study was that involuntary upper limb muscle contractions may have influenced our results. However, during each experiment, we ensured stabilization of the examined upper limb in a comfortable position. In addition, three incremental intensity levels were used to assess the impact of each exercise modality on brachial artery blood flow and shear rate. Such methodology minimized the potential influence of involuntary upper limb contractions on our results. Another potential limitation relates to the difference in “frequency” between cycling and walking (i.e., approximately 65 revolutions per steps per min) versus bilateral leg kicking (i.e., 20 kicks per min). Clinically, however, we found that a kicking “frequency” of 20 times per min was the maximum number achievable by the subject, without losing the quality of diameter and velocity recordings. Accordingly, our findings relate to bilateral leg kicking exercise at approximately 0.3 Hz specifically but not to a different kicking frequency or a different workload (e.g., high intensity). Such different kicking frequencies and/or workloads, which have been applied in previous studies to study acute (5) and chronic (3) effects of leg kicking exercise, may conceivably result in different brachial artery blood flow patterns, just as other differences in the elements of an exercise prescription (modality, frequency, duration, intensity) may induce different shear and blood flow patterns and arterial adaptations. Finally, gender is known to impact the change in blood flow and vascular conductance during forearm exercise (18,20). Because we studied only small groups of men and women (n = 6), we support future studies to focus on the possibility of gender differences in lower limb exercise-induced changes in the brachial artery blood flow pattern.

In summary, our results demonstrate that rhythmic cyclic lower limb exercise (e.g., cycling and walking) results in a reproducible shear rate pattern: a systolic anterograde shear rate, directly followed by a large retrograde shear during diastole. In contrast, a completely different type of exercise, such as leg kicking, is not associated with this pattern. Consequently, we have demonstrated that important differences exist in brachial artery flow and shear rate when comparing different lower limb exercise modalities. This raises the possibility that these differences in brachial artery blood flow and shear rate patterns may contribute to the distinct exercise training–induced vascular adaptations between these different exercise modalities.

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