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Shear Stress Mediates Endothelial Adaptations to Exercise Training in Humans

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Abstract—Although episodic changes in shear stress have been proposed as the mechanism responsible for the effects of exercise training on the vasculature, this hypothesis has not been directly addressed in humans. We examined brachial artery flow-mediated dilation, an index of NO-mediated endothelial function, in healthy men in response to an acute bout of handgrip exercise and across an 8-week period of bilateral handgrip training. Shear stress responses were attenuated in one arm by cuff inflation to 60 mm Hg. Similar increases were observed in grip strength and forearm volume and girth in both limbs. Acute bouts of handgrip exercise increased shear rate ($P<0.005$) and flow-mediated dilation percentage ($P<0.05$) in the uncuffed limb, whereas no changes were evident in the cuffed arm. Handgrip training increased flow-mediated dilation percentage in the noncuffed limb at weeks 2, 4, and 6 ($P<0.001$), whereas no changes were observed in the cuffed arm. Brachial artery peak reactive hyperemia, an index of resistance artery remodeling, progressively increased with training in the noncuffed limb ($P<0.001$ and 0.004); no changes were evident in the cuffed arm. Neither acute nor chronic shear manipulation during exercise influenced endothelium-independent glyceryl trinitrate responses. These results demonstrate that exercise-induced changes in shear provide the principal physiological stimulus to adaptation in flow-mediated endothelial function and vascular remodeling in response to exercise training in healthy humans. (Hypertension. 2010;55:00-00.)

Key Words: exercise ■ shear stress ■ nitric oxide ■ vascular function ■ arterial remodeling

Exercise training is a well-established and potent physiological stimulus which reduces primary1–3 and secondary cardiovascular events.4,5 Exercise training also improves nitric oxide (NO)-mediated endothelial function in coronary and skeletal muscle arteries of large and small caliber.6 Given its importance in a number of atherogenic processes,7–9 improvement in endothelial function may underlie some of the cardiovascular risk reduction associated with exercise training and physical activity.10

The mechanisms responsible for the benefits of exercise training in terms of endothelial function may be related to either direct hemodynamic effects, or secondary effects, mediated through risk factor modification. We have previously demonstrated that improvements in vascular function with exercise training can occur without change in traditional risk factor profiles,11 whereas exercise is known to be associated with acute changes in endothelial shear stress.12,13 It has therefore been proposed that episodic increases in shear stress may be the mechanism responsible for the beneficial impact of exercise training on vascular function.14,15 Indeed, increases in intraluminal shear stress improve NO-mediated endothelium-dependent dilation in animals.16,17 However, to our knowledge, this has not been directly addressed experimentally in humans.

In the present study, we took advantage of a within-subject design, involving simultaneous 8-week handgrip training of identical exercise intensity and duration of both forearms. During these supervised exercise bouts (30-minute, 4 times a week), shear stress was manipulated in one arm using cuff inflation, such that it remained near resting levels. We examined indices of endothelium-dependent artery function and remodeling at 2-week intervals over the 8-week training period. We hypothesized that changes in endothelial function and artery remodeling would be diminished in the limb exposed to lower shear stress stimulation during training.

Methods

Subjects
Ten healthy men (28±7 years) were recruited to examine the acute effects of handgrip exercise on brachial artery blood flow, shear pattern, and endothelial function in the noncuffed and cuffed arms. Another 11 healthy recreationally active male volunteers were recruited and allocated to an 8-week exercise training intervention (n=11) (Table 1).
Subjects were all young and healthy; none had 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. The study procedures were approved by the Ethics Committee of Liverpool John Moores University and adhered to the Declaration of Helsinki. Informed consent was gained from all participants before the experimental procedures.

Experimental Design

Initially, we examined the acute effects of a 30-minute handgrip exercise protocol. After measurement of preexercise baseline blood flow, shear pattern, and flow-mediated dilation (FMD) in both arms, we examined blood flow and shear patterns during bilateral handgrip exercise to establish that distinct shear stress stimuli existed in the cuffed versus noncuffed arms. FMD is a largely endothelium- and NO-dependent vasodilator response.18 It was reassessed after the 30-minute intervention in both arms to determine whether acute manipulation of shear stress during exercise modulates endothelial function (Figure 1).

To examine the impact of 8 weeks of handgrip training, subjects reported to the laboratory for initial assessment of anthropometric and vascular measurements. Assessment of FMD and vasodilator responses to ischemic exercise were taken at the beginning of the training program and then every 2 weeks until the end of an 8-week handgrip training period. Maximal voluntary contraction (MVC) was also assessed every 2 weeks, with forearm volume and girth assessed at the beginning and end of the 8-week training period. A schematic explaining the experimental design and procedures for the acute and chronic aspects of the experiment is provided in Figure 1.

Experimental Procedures

Acute Effects of Handgrip Exercise

In a recent article published in *Hypertension*, we compared the acute effects of handgrip exercise, cycle exercise, and forearm heating on FMD and GTN responses.12 In the present study, we extended the handgrip data from this experiment to provide a background for the impact of repeated exercise bouts, or exercise training. Subjects rested for 15-minute in a comfortable chair in the upright position in a quiet, temperature controlled room. Baseline bilateral brachial artery diameter and velocity were recorded using high-resolution duplex ultrasound for at least 1-minute, followed by assessment of brachial artery endothelial function using FMD, as described below. Subsequently, subjects performed bilateral handgrip exercise using identical dynamometers at a cadence of 30 contractions per minute for 30-minute (assisted by a metronome). While both arms were simultaneously exposed to identical handgrip exercise, a cuff was placed around one arm and inflated to 60 mm Hg. Placement of this cuff around the left or right arm was randomized between subjects. Brachial artery blood flow and shear were recorded during the handgrip exercise intervention in both the cuffed and noncuffed arms. Immediately after the 30-minute intervention period, bilateral brachial artery endothelial function was reassessed using FMD.

On a separate visit to the laboratory undertaken at the same time of day, subjects repeated the interventions described above, with the difference that in place of FMD assessments, we examined endothelium-independent NO-mediated dilation to sublingual administration of glyceryl trinitrate (GTN) (400 μg) before and after handgrip exercise (with and without cuff inflation).

![Figure 1. Schematic illustrating the experimental design and methods.](image)

**Table 1. Baseline Characteristics of Exercise Training Subjects (n=11) Before (0 Week) and After the Exercise Intervention (8 Week) in the Noncuffed and Cuffed Arm**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Noncuffed 0 Week</th>
<th>Noncuffed 8 Week</th>
<th>Cuffed 0 Week</th>
<th>Cuffed 8 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
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<tr>
<td>Weight, kg</td>
<td>82±12</td>
<td>82±12</td>
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</tr>
<tr>
<td>Height, cm</td>
<td>181±6</td>
<td>181±6</td>
<td>181±6</td>
<td>181±6</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128±10</td>
<td>128±10</td>
<td>128±10</td>
<td>128±10</td>
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<tr>
<td>DBP, mm Hg</td>
<td>59±6</td>
<td>59±6</td>
<td>59±6</td>
<td>59±6</td>
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<tr>
<td>MAP, mm Hg</td>
<td>83±7</td>
<td>83±7</td>
<td>83±7</td>
<td>83±5</td>
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<tr>
<td>HR, bpm</td>
<td>55±11</td>
<td>55±6</td>
<td>55±11</td>
<td>55±6</td>
</tr>
<tr>
<td>MVC, kg</td>
<td>42±10</td>
<td>54±9*</td>
<td>41±9</td>
<td>51±7*</td>
</tr>
<tr>
<td>Forearm volume, mL</td>
<td>1400±284</td>
<td>1472±250*</td>
<td>1401±283</td>
<td>1480±299*</td>
</tr>
<tr>
<td>Forearm girth, inch</td>
<td>27.8±0.6</td>
<td>28.6±0.6</td>
<td>27.3±0.7</td>
<td>28.0±0.7*</td>
</tr>
</tbody>
</table>

*Significant from pretraining at P<0.05 (paired t test). Values are means±SD. DBP indicates diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; MVC, maximal voluntary contraction; SBP, systolic blood pressure.

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**Figure 1.** Schematic illustrating the experimental design and methods. In A, methods used to assess acute responses to unilateral cuff placement during simultaneous assessment of blood flow and shear rate during, and FMD- and GTN-mediated dilation before and after, an acute bout of bilateral handgrip exercise. In B, the experimental design associated with assessment, at 2 weekly intervals, of FMD, GTN, and ischemic handgrip exercise across an 8-week intervention involving bilateral handgrip exercise training (3 times for 30 minutes weekly), where 1 forearm has a cuff (60 mm Hg) placed to arrest blood flow and shear rate responses during each exercise bout.
Cuff inflation to 60 mm Hg was selected on the basis of extensive pilot studies in which we measured the impact of different cuff pressures on the magnitude of antegrade and retrograde flow patterns and also on the basis of results from our previous experiment\(^{12}\) in which this intervention was effective in modifying blood flow and shear rate during the handgrip and FMD following hand grip exercise.

**Effects of Handgrip Training**

Exercise training was performed over an 8-week period with subjects visiting the laboratory 3 times a week and performing 1 session at home (see Figure 1). Each laboratory session was supervised and consisted of 30-minute of simultaneous handgrip exercise (30-contractions per min) at 30% MVC for 4 weeks, 40% MVC for 2 weeks, and the final 2 weeks at 50% MVC. Across the 8-week exercise training period, there was 90% adherence to the training sessions.

During each 30-minute training session, a pneumatic blood pressure cuff was placed below the cubital crease on one forearm of each subject and inflated to 60 mm Hg throughout the exercise period. The arm selected for cuff placement was randomized but, once selected, remained consistent for each subject across the 8-week training period.

**Experimental Measures**

Vascular assessments were conducted in a quiet, temperature controlled environment. Each visit for a given subject was performed at the same time of day. Subjects were asked to fast for >4 hours, abstain from alcohol and caffeine for 16 hours, and not to perform any exercise for 24 hours.

**Brachial Artery Endothelial Function**

After a 15-minute baseline rest, we examined bilateral brachial artery FMD. We used two 10-MHz multifrequency linear array probes, attached to high-resolution ultrasound machines (T3000; Terason, Burlington, Mass), to simultaneously assess diameter and velocity changes. A detailed description of this technique is provided elsewhere.\(^{12,19}\) Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap; GE Pro 300V2, Tampa, Fla).

**Brachial Artery Dilator Capacity**

Following a >15-minute rest period, we examined brachial artery dilation after 5-minute of ischemic exercise, described in detail elsewhere.\(^{20}\) This protocol results in dilation of the brachial artery, which is thought to be endothelium-dependent but not as highly dependent on NO release as the FMD approach described above.\(^{18}\) The peak hyperemic forearm blood flow response to this stimulus in humans provides a valid and accepted index of resistance artery size and remodeling, which has been used extensively the past 2 decades in previous human investigations.\(^{21,22}\)

**Brachial Artery Endothelium-Independent Vasodilation**

Following a >15-minute rest period, a 1-minute baseline recording of diameter, flow, and shear stress was taken from both limbs. Subsequently, brachial artery endothelium-independent vasodilation was examined after administration of a single spray of sublingual GTN (400 µg), an NO donor. This was followed by 10 minute continuous recordings of the diameter images in both arms.

**Anthropometry**

Maximal forearm girths were assessed using a Lufkin diameter tape (Lufkin, Mexico), and forearm volume in both arms was determined by immersion of the forearm to the cubital crease. Three measurements of girth and volume were taken on each arm and the mean was derived. MVC of both forearms was assessed as the mean of 3 measurements using a handgrip dynamometer (Stoelting, Wood Dale, Ill).

**Data Analysis**

**FMD and GTN**

FMD and GTN are presented as the absolute (mm) and relative (%) rise from the preceding baseline diameter and are calculated based on standardized algorithms applied to data that had undergone automated edge detection and wall tracking and were therefore observer-independent.\(^{24}\) See previous studies for further detail.\(^{24}\)

In accordance with recent findings,\(^{24,27}\) we calculated the shear rate stimulus responsible for endothelium-dependent FMD following cuff deflation. The area under the shear rate curve (AUC), calculated for data up to the point of maximal postdeflation diameter (FMD),\(^{24}\) was calculated for each individual.

Peak blood flow, calculated from diameter (cross-sectional area) and velocity data, was recorded as the highest area under the blood flow response to an incremental 50% isometric handgrip contraction following administration of GTN (400 µg) sublingually.
flow curve data across a 10-second period following each ischemic handgrip stimulus.20

Statistics

Statistical analyses were performed using SPSS 15.0 (SPSS, Chicago, Ill) software. All data are reported as mean (SD) unless stated otherwise, and statistical significance was assumed at P<0.05. Repeated-measures ANOVA (with time and group as independent factors) and post hoc analysis (with Tukey’s and correction for multiple comparisons) were used to assess the changes in brachial artery function, conduit artery dilator response to ischemic exercise, and peak forearm blood flow throughout the 8-week intervention period in the trained as well as in the control group.

Results

Acute Effect of Handgrip Exercise

Baseline brachial artery mean, antegrade, and retrograde shear rate (SR) and FMD% were similar between the limbs (t tests: P=0.97, P=0.98, P=0.79, and P=0.63, respectively; Figure 2). Handgrip exercise induced a significant increase in mean, antegrade, and retrograde SR in the noncuffed arm and these changes were associated with a significant increase in FMD% (Figure 2). Despite performing identical exercise with the contralateral limb, cuff inflation to 60 mm Hg resulted in no change in SR or FMD% (Figure 2). Attenuation of shear stress during exercise therefore abolished the increase in brachial artery FMD%.

The 30-minute intervention period did not alter GTN responses in either the noncuffed (n=6; pre: 16.5±2.8 and post: 15.5±7.3; P=0.71) or cuffed limbs (pre: 17.9±2.2; post: 17.3±5.9; P=0.75).

Chronic Effects of Eight Weeks of Handgrip Training

There were no significant differences at baseline between the arms in terms of girth, volume or strength (Table 1). Maximal voluntary contraction increased significantly in both arms after 8 weeks of handgrip training (P<0.001), and both limbs also demonstrated a similar increase in forearm volume and girth (P<0.05). Baseline brachial artery diameter did not change in either arm across the 8-week training period (Table 2).

Flow-Mediated Vasodilation

Localized handgrip training induced a significant change in brachial artery FMD% in the noncuffed arm (ANOVA, P=0.001; Figure 3A). Brachial artery FMD% values at 2, 4, and 6 weeks were significantly higher compared with baseline but returned to near baseline values at week 8 (Figure 3A). In the cuffed arm, no changes in FMD% were evident at any time point (ANOVA, P=0.83; Figure 3A). There was a significant interaction between limbs and across the time points over the 8 weeks of training (Figure 3A). No effect for time, limb, or interaction between these factors was evident for shear stress, calculated from cuff deflation to the point of peak diameter (AUCSR) (Table 2).

Vasodilator Response to Ischemic Exercise

In the noncuffed arm, the brachial artery dilator responses to ischemic exercise showed a progressive increase across the training intervention period (Figure 3B) (ANOVA, P=0.001). Peak blood flow also progressively increased across time in the noncuffed arm (ANOVA, P=0.001, Table 2; Figure 4). In the cuffed arm, no changes in brachial artery dilator response to ischemic exercise or peak blood flow were evident across the 8-week training intervention (Table 2; Figures 3B and 4).

Endothelium-Independent Vasodilation

In the noncuffed arm, brachial artery GTN responses did not change across the 8-week handgrip training (Table 2). Similarly, no changes in brachial artery dilation to GTN were evident in the cuffed arm across the 8-week training intervention (Table 2).

Table 2. Brachial Artery Characteristics Throughout an 8-Week Exercise Intervention in the Noncuffed and Cuffed Arms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Noncuffed arm</th>
<th>Cuffed arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter, mm</td>
<td>4.1±0.5</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>FMD, %</td>
<td>4.6±1.4</td>
<td>4.6±1.4</td>
</tr>
<tr>
<td>AUCSR (mm²/s)</td>
<td>2.4±0.8</td>
<td>2.4±0.8</td>
</tr>
<tr>
<td>Ischemic exercise dilation, %</td>
<td>10.1±3.4</td>
<td>10.1±3.4</td>
</tr>
<tr>
<td>Peak RH flow</td>
<td>683±2965</td>
<td>683±2965</td>
</tr>
<tr>
<td>Peak RH conductance</td>
<td>7.3±3.2</td>
<td>7.3±3.2</td>
</tr>
<tr>
<td>GTN, %</td>
<td>14.4±4.9</td>
<td>14.4±4.9</td>
</tr>
</tbody>
</table>

Significant from baseline at *P<0.05 or †P<0.005. Values are means ± SD. AUCSR indicates shear rate area under the curve; FMD, flow-mediated dilatation; RH, peak reactive hyperemic response to ischemic exercise.
Figure 3. Relative change in brachial artery flow mediated dilation from baseline (FMD%) (A) and in response to ischemic exercise (B) across the 8-week handgrip exercise training in healthy young men (n=11). Data are presented for the noncuffed arm (●), as well as the cuffed arm (□). Data are presented before, after, and at 2-week intervals throughout the 8-week intervention. Error bars represent SEM. Post hoc significantly different between the cuffed and noncuffed arm at *P<0.05.

Discussion

In the present study, we examined the contribution of shear stress to vascular adaptation in response to acute and chronic handgrip exercise using bilateral and simultaneous measurement of flow-mediated and endothelium-dependent vasodilator function. Although large increases in antegrade blood flow and shear stress were observed during handgrip exercise in the noncuffed arm, we effectively maintained antegrade flow and shear stress at near baseline levels within each subject in the contralateral exercising limb by cuff inflation to 60 mm Hg around 1 forearm throughout each training session. The principal findings of the present study were that: (1) a period of 30-minute of handgrip exercise is associated with an acute increase in FMD; (2) this increase in arterial function in response to acute exercise is abolished if not accompanied by an increase in shear stress; (3) 8 weeks of localized exercise training induces a time-dependent change in arterial function (ie, FMD); (4) 8 weeks of localized exercise training induces a time-dependent change in resistance vessel arterial remodeling; and (5) handgrip exercise training that induces improvements in grip strength and forearm volume does not induce changes in arterial function or remodeling if it is not associated with increases in shear stress. We have therefore established that an increase in FMD following an acute bout of handgrip exercise is dependent on increased shear stress. In addition, if repeated bouts of handgrip exercise (ie, training) are performed, time-dependent changes in both arterial function and remodeling are evident, which are also shear stress–dependent.

In the present study, we manipulated the magnitude of antegrade and retrograde flow and shear during handgrip exercise using cuff inflation. In the noncuffed arm, significant elevations in antegrade blood flow and shear were evident. However, antegrade flow and shear remaining near resting values in the cuffed arm. Interestingly, these differences in shear during handgrip exercise result in a different impact on the NO-mediated vasodilator function between arms. A significant increase in FMD% was observed in the noncuffed arm after handgrip exercise, which reinforces some recent findings that acute changes in shear stress can modulate the FMD%.12,26 However, the increase in FMD% was completely abolished in the cuffed arm. These findings therefore suggest that an increase in antegrade flow and shear is an important stimulus for acute enhancement of endothelial function in vivo.

Localized exercise training in healthy subjects improves strength, girth, and volume, but studies have not consistently reported improvement in NO-mediated endothelial function after such training.28–30 A potential explanation is that exercise is initially associated with functional adaptations, which are subsequently superseded by other adaptations, allowing NO-mediated function to return toward baseline levels. There is some evidence in animals which supports this notion of a time course of adaptation in artery function.31–33 Interestingly, our repeated measures design allowed us to demonstrate that brachial artery NO-dependent function initially increased in response to handgrip training but returns to near resting values after a longer training period. The change in function was superseded by a gradual increase in peak reactive hyperemia, a frequently used and accepted method to examine resistance vessel remodeling20–22 across the 8 weeks of training. The time-dependent normalization of brachial artery endothelial function raises the possibility that, depending on
the time of (re)assessment, previous studies that have not demonstrated an effect of prolonged exercise training on NO-mediated vasodilator function may conceivably have missed the response by measuring it at a limited number of time points.\textsuperscript{28,30} The present finding relating to the time course of functional adaptation is also consistent with our recent report of brachial and popliteal changes in function across 8 weeks of lower-limb exercise training.\textsuperscript{34} Further research will be required to specifically address the question of whether structural remodeling, which is NO-dependent,\textsuperscript{35} may supersede changes in vascular function, is associated with the return of arterial function to resting values.\textsuperscript{31,32}

Interestingly, although there have been studies in both animals\textsuperscript{35} and humans\textsuperscript{45} that suggest that functional and remodeling vascular adaptations to training are shear stress dependent, this study is the first, to our knowledge, to manipulate shear during exercise in healthy subjects. In response to training, the cuffed arm (in which shear was manipulated and remained near resting values) demonstrated significant increases in forearm strength, volume, and girth, which were similar in magnitude to the noncuffed arm. However, the cuffed arm displayed no change in NO-mediated function or resistance artery remodeling across the 8-week handgrip training period. In contrast, the noncuffed arm, in which exercise was associated with increases in antegrade flow and shear, showed increases in both FMD\% and peak hyperemia responses over the 8-week handgrip training period. These data strongly implicate changes in arterial shear stress in the transduction of exercise mediated vascular effects.

Ischemic handgrip resulted in a larger shear stress and brachial artery dilution response than the FMD stimulus in the present study. Ischemic handgrip is therefore a stimulus similar to those used by Mullen et al\textsuperscript{18}; that is, shear stress present study. Ischemic handgrip is therefore a stimulus larger than the FMD stimulus in the vascular effects.

Arterial shear stress in the transduction of exercise mediated and peak hyperemia responses over the 8-week handgrip training period. In contrast, the noncuffed arm, which was therefore exposed to any

reflex or hemodynamic changes, because of our control of central nervous system function. The impact of large muscle group exercise, as distinct from handgrip training, on shear stress–mediated adaptations to training will be an interesting subject for future investigation.

**Perspectives**

Our results demonstrate that exercise-induced increases in shear provide a potent physiological stimulus to adaptation in endothelial function and vascular remodeling in response to exercise training in healthy humans. Although inflation of a cuff on one forearm throughout the 8 weeks of training abolished the acute and chronic shear stress stimulus and consequent vascular adaptations present in the noncuffed arm, simultaneous bilateral improvements were observed in muscle strength, forearm girth, and volume after exercise training. These results provide further insight into the time course of both vascular adaptations associated with exercise but, more importantly, highlight shear stress as a principal mechanism responsible for exercise-induced vascular adaptations in function and structure.

**Acknowledgments**

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**Disclosures**

None.

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