Brachial artery adaptation to lower limb exercise training: role of shear stress

Gurpreet K. Birk, Ellen A. Dawson, Ceri Atkinson, Andrew Haynes, N. Timothy Cable, Dick H. J. Thijsse, and Daniel J. Green

1Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom; 2Department of Physiology, Radboud University Nijmegen Medical Centre, The Netherlands; 3School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia

Submitted 5 December 2011; accepted in final form 29 February 2012

EXERCISE TRAINING HAS POTENT cardioprotective effects that are not fully explained by modification of traditional or novel cardiovascular risk factors (9, 29). The beneficial effects of exercise training may partly be mediated through direct effects of exercise on the vasculature (17, 24). It has been reported that exercise training leads to localized improvements in vascular function and structure in the active regions (50), which may be mediated through repetitive increases in shear stress (31, 49, 50). Exercise training also results in systemic vascular adaptations beyond the active regions (10, 27, 28). However, to date, relatively little is known about the hemodynamic stimuli responsible for systemic effects of large muscle group exercise training on the vasculature.

One potential explanation for systemic vascular adaptations to exercise training relates to shear stress. We have demonstrated that shear increases in the inactive upper limbs in a "dose-dependent" manner during leg cycling (11, 14, 44) and others have recently confirmed these observations (33, 34, 42). Although the average increase in shear rate (SR) in the upper limbs is smaller than that observed in the active lower limbs (44), or in response to handgrip exercise (11), this can mask substantial changes in the flow and shear that may contribute to vascular adaptations in the nonactive regions as a result of training (49). Indeed, recent observations indicate that brachial artery mean shear rate progressively increases during constant load leg cycling exercise, most likely as a consequence of cutaneous thermoregulatory vasodilation (41). These findings suggest the presence of a significant brachial artery stimulus during lower limb exercise. Therefore, the purpose of this study was to examine upper limb brachial artery endothelium-dependent and -independent dilation before, during, and after 8 wk of lower limb cycling training in healthy humans.

To specifically address the role of shear, subjects exercised with a cuff around one forearm to unilaterally manipulate the exercise-induced increases in this variable (13, 31, 49, 50). We hypothesized that cycle exercise training would lead to improvement in endothelial function in the nonactive upper limb, in keeping with time-dependent changes observed in recent exercise training studies in healthy humans (48, 50) and that such adaptation would be shear stress mediated.

METHODS

Subjects

Eleven healthy men (22 ± 2 yr) were recruited to examine the effects of 8-wk cycle exercise on brachial artery endothelial function in the noncuffed and cuffed arms. Subjects were healthy; none reported having been diagnosed with cardiovascular diseases, diabetes, insulin resistance, or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects were recreationally active (1–5 h of physical activity per week). Informed consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

Experimental Design

Effect of upright cycle ergometer exercise on brachial artery shear stress: impact of forearm cuff inflation. Five additional healthy male subjects (24 ± 2 yr), distinct from those studied above, were recruited to undertake 30 min of cycle exercise (Monark 874E, Sweden) at 80% maximal heart rate (80%HRmax; SWY) throughout this exercise bout, a pneumatic cuff was placed around one forearm immediately below the cubital crease and inflated to 60 mmHg. The contralateral arm remained uncuffed during cycle exercise. Brachial artery diameter and velocity values were simultaneously collected immediately prior to exercise (before cuff inflation) and at 25 min during exercise with the cuff inflated. Briefly, subjects rested for 20 min while seated at rest on...
a stationary bicycle ergometer in a quiet, temperature-controlled room. Baseline bilateral brachial artery diameter and velocity were recorded using high-resolution duplex ultrasound for at least 1 min. Subsequently, subjects performed leg cycling exercise for 30 min at 80%HR_{max}. Brachial artery blood flow and shear were recorded during the leg cycling exercise intervention in both the cuffed and noncuffed arms (Fig. 1). Previous studies have demonstrated that placement and inflation of a forearm cuff in this manner attenuates upstream brachial artery shear rate (13, 31, 49, 50). We previously published examples of images acquired both at rest and during exercise (47, 51).

**Effect of cycle ergometer training on brachial artery adaptation.** Exercise training was performed over an 8-wk period with subjects visiting the laboratory three times per week. Each laboratory session was supervised and consisted of 30-min of cycle exercise (80%HR_{max}) performed at the same time of day. During each 30-min training session, a pneumatic blood pressure cuff was placed below the cubital crease on one forearm and inflated to 60 mmHg throughout the exercise period. Cuff inflation to 60 mmHg was selected on the basis of previous experimentation (13, 31, 49, 50) in which unilateral cuff inflation was effective in modifying blood flow and shear rate during exercise. Subjects were instructed not to hold the handle bars to ensure inactivity of the upper limbs during cycling exercise. The arm selected for cuff placement was randomized, but once selected remained consistent for each subject across the 8-wk training period.

To examine the role of shear rate in systemic vascular adaptation to 8-wk of leg cycle training, we examined bilateral baseline FMD%, brachial artery diameter response to an ischemic exercise stimulus (iEX), described in detail elsewhere (32). This protocol results in dilation of the brachial artery, which is considered endothelium dependent, but may not be highly NO mediated (1, 6, 10, 30).

**Brachial artery responses to ischemic exercise.** Following a further 20-min rest period, we examined brachial artery flow-mediated dilation (FMD) in both arms. Bilateral measures were collected simultaneously to minimize error. We used two 10-MHz multifrequency linear array probes, attached to high-resolution ultrasound machines with identical settings (T3000; T器ason, Burlington, MA), to simultaneously assess diameter and velocity changes. A detailed description of this technique is provided elsewhere (4, 49). This approach elicits endothelium-dependent and largely nitric oxide (NO)-mediated dilation (10). Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap; GE Pro 300V2, Tampa, FL).

**Brachial artery responses to ischemic exercise.** Following a further 20-min rest period, we examined brachial artery dilation after an ischemic exercise stimulus (iEX), described in detail elsewhere (32). This protocol results in dilation of the brachial artery, which is considered endothelium dependent, but may not be highly NO mediated (1, 6, 10, 30).

**Brachial artery endothelium-independent vasodilation to glyceryl trinitrate.** Following another 20-min rest period, a 1-min baseline recording of diameter, flow, and shear rate was taken from both limbs. A single spray of sublingual glyceryl trinitrate (GTN, 400 μg), a NO donor, was then administered followed by a 10-min continuous recordings of the diameter images in both arms to determine brachial artery endothelium-independent vasodilation.

**Brachial Artery Diameter and Blood Flow Analysis**

Posttest analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software that is largely independent of investigator bias (51). Recent papers contain detailed descriptions of our analysis approach (3). From synchronized diameter and velocity data, blood flow [the product of lumen cross-sectional area and Doppler velocity (V)] was calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as four times mean blood velocity/vessel diameter (36). Reproducibility of the FMD using this semi-automated software possesses a coefficient of variation of 6.7–10.9% (45).

**Data Analysis**

FMD%, iEX, and GTN are presented as the relative (%) rise from the preceding baseline diameter and are calculated based on standardized algorithms applied to data that had undergone automated observer-independent edge-detection and wall-tracking [see previous studies for further detail (3)]. In accordance with recent findings (3, 37), we calculated the shear rate stimulus responsible for endothelium-dependent FMD% following cuff deflation. The area under the shear rate curve (SR_{AUC}), calculated for data up to the point of maximal postdeflation diameter (FMD%\(3\)), was calculated for each individual. Peak blood flow in response to the FMD test was determined as the highest flow in the first 20 s after deflation, using bins of ~3 s while ensuring entire cardiac cycles were included. This shear rate response is exclusive for the FMD% tests and does not relate to the shear responses during exercise bouts. In accordance with recent guidelines (43), we presented the FMD% SR data in a table, but have not normalized for this data due to the limitations of this approach (2).

---

Fig. 1. Brachial artery mean, antegrade, and retrograde shear rate during rest and cycle exercise in the noncuffed (A) and cuffed (B) arms of healthy, young volunteers (n = 5). Post hoc t-test significantly different from pre (†) and from cuffed (*) at P < 0.05.
The ratio between FMD to GTN was also calculated and provides an assessment of the NO dilator system, whereby endothelial NO function is presented in the context of smooth muscle cell sensitivity to NO (20, 21, 35, 38).

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. All data are reported as mean (SD) unless stated otherwise, whereas statistical significance was assumed at \( P < 0.05 \). Initially, a two-factor ANOVA with repeated measures (with time and cuff placement as independent factors) was performed for the initial acute blood flow data. Additional, repeated-measures ANOVA (with time and cuff placement as independent factors) were used to assess changes in brachial artery vasodilation in response to FMD% (i.e., primary outcome parameter), iEX, and GTN across the 8-wk intervention period. Post hoc analysis \( t \)-tests were used where significant values were found.

RESULTS

Acute Effects of Leg Cycling Exercise

Mean (arm = 0.003, time = 0.005, arm*time = 0.005) and antegrade (arm = 0.015, time = 0.007, time*arm = 0.031) shear rate significantly increased from baseline in both arms \( (P < 0.05) \). Mean \( (P = 0.004) \) and antegrade \( (P = 0.019) \) SR were both significantly larger in the noncuffed vs. cuffed arm during exercise (Fig. 1; \( P < 0.05 \)). In addition brachial artery retrograde shear rate was significantly larger in the cuffed, compared with the non-cuffed arm (Fig. 1), during exercise \( (P = 0.017) \).

Chronic Effects of 8-wk Leg Cycling Exercise Training

Two subjects were unable to complete the study for personal reasons unrelated to the study or its protocols. Across the 8-wk exercise training period, there was 91.7% adherence to the training sessions in the remaining nine subjects. There were no significant changes in mean arterial blood pressure \( (87 \pm 6, 86 \pm 7, 87 \pm 4, \text{and} 85 \pm 6, \text{ANOVA}: P = 0.81) \) or heart rate \( (64 \pm 3, 61 \pm 9, 57 \pm 7, \text{and} 60 \pm 7, \text{ANOVA}: P = 0.09) \) between weeks 0, 2, 4, and 8 of leg cycle training. There were no differences in baseline brachial artery characteristics between arms at week 0 (Table 1).

**Brachial artery FMD responses to ischemia.** Cycle exercise training induced a significant increase in brachial artery FMD% \( (\text{ANOVA}, P = 0.04; \text{Fig. 2A}) \). Post hoc analysis revealed that brachial artery FMD% in the noncuffed arm increased at week 2 \( (P < 0.05 \text{ vs. } \text{week 0}) \), before returning to near baseline values at weeks 4 and 8 \( (\text{Fig. 2A}) \). In the cuffed arm, no changes in FMD% were evident at any time point (Fig. 2A). No change was evident across the 8-wk training period in the elicited shear stress stimulus \( (\text{SR}_{\text{AUC}}) \) to FMD%, baseline diameters, or time-to-peak dilation (Table 1). There was also no difference between the limbs or across time in peak blood flow responses to the FMD test.

**Brachial artery responses to iEX.** In the noncuffed and cuffed arms, the brachial artery dilator responses to iEX did not significantly differ across the training intervention period \( (\text{ANOVA}, P = 0.56; \text{Table 1}) \).

**Endothelium-independent, NO-mediated dilation (GTN) responses.** Leg cycling exercise training induced a significant change in brachial artery GTN by two-way ANOVA \( (\text{time factor } P < 0.01; \text{Fig. 2B}) \). Post hoc analysis revealed nonsignificant decreases in GTN responses between weeks 0, 2, and 4, with a significant increase in GTN in both arms between week 4 and 8. Nonetheless, no significant change in GTN was apparent in either arm when week 8 and week 0 data were directly compared.

**FMD/GTN.** We calculated the ratio of FMD to GTN responses to reflect changes in endothelial function, normalized to smooth muscle adaptation. This data reinforced the FMD% results in that the ratio in the noncuffed was significantly increased at weeks 2 and 4 \( (\text{ANOVA}: P < 0.01; \text{Fig. 2C}) \), relative to week 0, with week 8 data not significantly different from baseline. No significant changes were apparent in the cuffed limb (Fig. 2C).

DISCUSSION

The purpose of the present study was to determine the impact of shear rate on brachial artery endothelium-dependent and -independent function in response to lower limb cycle ergometer training. Our principal finding was that a rapid increase in endothelium-dependent and largely NO-mediated vasodilator function was apparent in the limb exposed to

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 8</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter, mm</td>
<td>Cuffed</td>
<td>3.7 ± 0.6</td>
<td>3.6 ± 0.8</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Noncuffed</td>
<td>3.8 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>FMD% time to peak, ( \text{s} )</td>
<td>Cuffed</td>
<td>44 ± 13</td>
<td>46 ± 16</td>
<td>55 ± 23</td>
<td>43 ± 16</td>
</tr>
<tr>
<td></td>
<td>Noncuffed</td>
<td>57 ± 20</td>
<td>49 ± 17</td>
<td>49 ± 14</td>
<td>63 ± 37</td>
</tr>
<tr>
<td>FMD% SRauc, ( \text{10}^3, \text{ s}^{-1} )</td>
<td>Cuffed</td>
<td>17.7 ± 7.7</td>
<td>21.6 ± 13.9</td>
<td>19.6 ± 7.7</td>
<td>20.6 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>Noncuffed</td>
<td>17.9 ± 9.0</td>
<td>21.2 ± 7.1</td>
<td>23.2 ± 11.3</td>
<td>20.1 ± 9.7</td>
</tr>
<tr>
<td>FMD% peak BF, \text{ml/min}</td>
<td>Cuffed</td>
<td>422 ± 153</td>
<td>390 ± 167</td>
<td>396 ± 153</td>
<td>449 ± 116</td>
</tr>
<tr>
<td></td>
<td>Noncuffed</td>
<td>404 ± 128</td>
<td>510 ± 138</td>
<td>474 ± 144</td>
<td>453 ± 228</td>
</tr>
<tr>
<td>iEX, %</td>
<td>Cuffed</td>
<td>16.3 ± 5.7</td>
<td>14.8 ± 4.5</td>
<td>14.5 ± 7.0</td>
<td>14.3 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Noncuffed</td>
<td>12.8 ± 4.3</td>
<td>12.9 ± 4.0</td>
<td>14.3 ± 4.7</td>
<td>12.0 ± 5.2</td>
</tr>
</tbody>
</table>

Values are means ± SD, FMD, flow-mediated dilatation; iEX, ischaemic handgrip exercise, GTN; glyceryl trinitrate; SRauc, shear rate area underneath curve; peak BF, peak blood flow.

\[ \text{doi:10.1152/japplphysiol.01489.2011 \ www.jappl.org} \]
increased shear stress during leg training, but not in the contralateral cuffed limb in which shear was attenuated. Changes in FMD% in the noncuffed arm were transient, returning to near baseline values at week 8. When FMD responses were normalized for changes in smooth muscle sensitivity, the FMD/GTN ratios also revealed significant increases in artery function in the noncuffed arm between weeks 0, 2, and 4, with no differences between weeks 0 and 8. These data suggest that lower limb exercise induces vascular functional changes evident in the untrained upper limbs, which are attributable, at least in part, to the systemic effects of lower limb exercise on shear stress.

In the present study, cycle exercise training induced endothelial adaptations in brachial artery of the noncuffed arm only. This is in line with recent studies that examined the impact of exercise training (50) and bilateral forearm heating (13, 31) in the presence and absence of cuff inflation. Collectively, these studies suggest that shear stress is a key stimulus responsible for training-induced adaptation in conduit artery function in humans. The transient nature of the effect, with resolution by 8 wk, has been previously described and discussed (31, 50) and suggests that healthy subjects demonstrate improvement in endothelial function during initial phases of training that returns toward baseline as exercise training continues. This phenomenon was first suggested by Laughlin and colleagues (26) on the basis of extensive animal data, and it may explain the lack of effect observed in training studies of healthy subjects, which collected single point, rather than time-course data (5, 12).

In our previous study of handgrip exercise training (18), the return of FMD% to baseline levels was accompanied by increases in iEX-mediated dilation, suggesting either that functional changes are superseded by structural remodeling or that adaptation in endothelial function other than that associated with NO may occur over time. However, in the present study, no changes in GTN or iEX-mediated dilation were apparent at 8 wk. Although we cannot exclude the possibility that late changes occur in the function or remodeling of upper limb arteries in response to lower limb cycle exercise, this seems unlikely based on the findings of the current study, and there is limited evidence for systemic effects of exercise on arterial remodeling in humans (16, 40). Indeed, comparison of specific groups of upper or lower limb-dominant athletes (22), including the preferred and nonpreferred limbs of racquet sportsmen (15, 39), indicate that changes in artery size predominantly occur in response to local, rather than systemic, stimuli. This may suggest that the magnitude and/or pattern of shear stress in the brachial artery in response 8 wk of lower limb exercise is insufficient to induce structural adaptation, despite the evidence we present for initial changes in endothelial function.

In a previous paper, Tanaka et al. (42) found that leg cycling exercise results in a gradual and curvilinear increase in brachial artery shear rate, leading to a fourfold increase in shear. In our paper, a ~4-fold increase was also evident in brachial artery shear in the uncuffed arm. In the cuffed arm, although brachial artery shear rate was lower compared with the noncuffed arm, shear rate still increased ~2-fold during exercise. Nonetheless, no changes in endothelial function were found. These data suggest that a shear stress threshold may be necessary to induce adaptations, a finding that we also suggested on the basis of our handgrip training study (50). Future studies will be necessary to examine this hypothesis.

The observation that exercise induces shear stress-mediated changes in NO signal transduction is not new. In humans, Hambrecht (19) demonstrated upregulation of eNOS mRNA and protein, including shear stress sensitive phosphorylation components of the enzyme. Although this paper linked exercise and shear stress to eNOS adaptation, it did not directly manipulate or measure shear stress. Other studies have reported that leg exercise training can induce chronic changes in upper limb resistance and conduit artery function (7, 16, 28). However, our findings are novel in that they provide the first evidence in humans that exercise of large muscle groups, associated with hemodynamic changes in blood pressure and cardiac output, is also associated with systemic changes in artery function that are mediated by shear stress.

We characterized mean brachial artery shear stress in the cuffed and noncuffed limbs during leg cycle ergometry in the present study and also the antegrade and retrograde compo-
tions that increase retrograde shear can be associated with systemic changes in neural activation could explain the unilat-
eral impacts of training that we observed. Similarly, circulating hormonal impacts of training would, logically, have elicited functional impacts during cycling training, our data nonetheless emphasize the importance of shear rate patterns on systemic arterial adaptation.

Shear stress has already been identified as a key stimulus for local exercise-induced vascular adaptations. We extend this knowledge by providing evidence that systemic effects of exercise training are, at least partly, mediated via shear stress-dependent mechanisms. This direct effect of shear on the vessel wall may contribute to the well-known cardioprotective effects of exercise training (17, 24). These observations may lead to future studies that manipulate exercise-induced elevations in shear, for example by additional heat stimuli, to further optimize the effects of exercise training. Along these lines, previous studies have found beneficial effects of local (forearm heating) or systemic (sauna therapy) application of heat to improve endothelial function in healthy (31) and diseased groups (23, 25).

Several limitations in this study are germane. This study involved young healthy male volunteers and we cannot extrapolate our findings to subjects with cardiovascular disease or older individuals or women. Vascular function is affected by the sympathetic nervous system, but it seems unlikely that systemic changes in neural activation could explain the unilateral impacts of training that we observed. Similarly, circulating hormonal impacts of training would, logically, have elicited bilateral changes in artery function, as would also have been the case for direct effects of blood pressure on functional arterial adaptation. Finally, we did not assess all vasodilator or constrictor pathways, and it is possible that functional adaptations that superseded the FMD response were missed.

Conclusion

Findings from the present study indicate that systemic adaptations in flow-mediated endothelial function occur in the nonexercising upper limbs as a result of leg cycle exercise training. This response is at least partly explained by the impact of leg cycling on brachial artery blood flow and shear stress, as vascular adaptations were abolished when shear stress was attenuated through cuff inflation of the forearm during cycling. Not only do these data reinforce the time course of vascular adaptations following exercise training, they also provides further insights into the role of increases in blood flow and shear stress as a stimulus for systemic vascular adaptations. To our knowledge, data from the present study provide evidence for the first time of the systemic effect of exercise training via shear stress-dependent mechanisms. Taken together these data provide evidence for an intervention capable of eliciting systemic vascular benefits in humans.

ACKNOWLEDGMENTS

We thank Niamh Digby-Bratton, Christie Quixall, Jessica Holmes, and Emma Roberts for assistance during the assessments and our subjects for participation during the 8 wk of exercise training.

GRANTS

Dr Thyssen is recipient of the E. Dekker stipend (Netherlands Heart Foundation, 2009T064). Prof. Green is funded by the National Heart Foundation of Australia and the Australian Research Council.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


