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Abstract—Shear stress is an important stimulus to arterial adaptation in response to exercise and training in humans. We recently observed significant reverse arterial flow and shear during exercise and different antegrade/retrograde patterns of shear and flow in response to different types of exercise. The purpose of this study was to simultaneously examine flow-mediated dilation, a largely NO-mediated vasodilator response, in both brachial arteries of healthy young men before and after 30-minute interventions consisting of bilateral forearm heating, recumbent leg cycling, and bilateral handgrip exercise. During each intervention, a cuff inflated to 60 mm Hg was placed on 1 arm to unilaterally manipulate the shear rate stimulus. In the noncuffed arm, antegrade flow and shear increased similarly in response to each intervention (ANOVA; $P<0.001$, no interaction between interventions; $P=0.71$). Baseline flow-mediated dilation (4.6%, 6.9%, and 6.7%) increased similarly in response to heating, handgrip, and cycling (8.1%, 10.4%, and 8.9%, ANOVA; $P<0.001$, no interaction; $P=0.89$). In contrast, cuffed arm antegrade shear rate was lower than in the noncuffed arm for all of the conditions ($P<0.05$), and the increase in flow-mediated dilation was abolished in this arm (4.7%, 6.7%, and 6.1%; 2-way ANOVA: all conditions interacted $P<0.05$). These results suggest that differences in the magnitude of antegrade shear rate transduce differences in endothelial vasodilator function in humans, a finding that may have relevance for the impact of different exercise interventions on vascular adaptation in humans. **(*Hypertension*. 2009;54:278-285.)**

Key Words: conduit artery ■ flow-mediated dilation ■ exercise training

Exercise training is a well-established and potent physiological stimulus that reduces primary[1–3] and secondary cardiovascular events.[4,5] Improvement in endothelial function induced by exercise training may contribute to these beneficial effects in cardiovascular risk.[6] Data in animals and humans suggest that endothelial shear stress is a key stimulus responsible for vascular adaptation in both artery function and remodeling in response to repeated exercise.[7–9] However, little is known about the exact shear stress stimulus responsible for the beneficial exercise-induced vascular adaptations.

We demonstrated recently that shear rate (SR) in the brachial artery differs markedly in response to different types of exercise. For example, handgrip exercise induces an elevation in antegrade SR, whereas cycling results in large increases in antegrade and retrograde blood flow and SR.[10] The observation that different types of shear are present during various types of exercise raises the question of whether different patterns of SR are associated with different vascular adaptations. Although studies performed in vitro and in animals have suggested that different shear patterns induce different cellular events, varying between proatherogenic and antiatherogenic changes,[9] limited information is available in humans.

The primary purpose of our study was to examine whether different flow and shear stimuli mediate different acute changes in vascular function, examined using flow-mediated dilation (FMD), a largely endothelium- and NO-dependent stimulus,[11–15] in humans. We measured brachial artery FMD before and after 3 different 30-minute interventions (recumbent leg cycling, forearm heating, and handgrip exercise) that were associated with significantly different SR patterns. To further elaborate on the impact of blood flow and SR patterns on endothelial function, we simultaneously performed identical interventions in the contralateral limb of each subject, which had a cuff inflated to 60 mm Hg throughout the intervention period to attenuate shear levels within subjects.

Methods

Subjects

Ten healthy, recreationally active male volunteers were recruited (28±7 years; Table 1), and informed consent was obtained from all

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Table 1. Baseline Characteristics of Healthy Young Subjects Before (Pre) and After (Post) Each Intervention

	Forearm Heating		Handgrip		Recumbent Leg Cycling		
Parameter	Pre	Post	Pre	Post	Pre	Post	ANOVA *P*
SBP, mm Hg	132±18	131±13	123±18	131±17*	122±17	117±17	0.08
DBP, mm Hg	56±10	55±9	51±9	53±12	54±30	48±14	0.76
MAP, mm Hg	81±11	80±10	75±12	78±13	76±25	71±13	0.39
HR, bpm	56±8	62±11	59±11	55±11	60±9	84±3*	0.59

Values are mean±SD. The *P* value refers to 1-way repeated-measures ANOVA between the preintervention values. No differences were found between pretest values among the 3 testing days. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

*Data are significant from pre-exercise at $P<0.05$ (paired *t* test).

of the participants before the experimental procedures. Subjects were healthy; none reported having been diagnosed with cardiovascular disease, diabetes mellitus, insulin resistance, or cardiovascular risk factors, eg, 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.

Experimental Design

Subjects reported to the laboratory on 3 occasions to perform distinct 30-minute interventions consisting of bilateral forearm heating, recumbent leg cycling, or bilateral handgrip exercise. Previous studies suggested that these interventions should result in different brachial artery blood flow patterns.[16,17] By adjusting the intensity of each exercise bout, we matched brachial artery mean SR, within subjects, to that observed during exposure to forearm heating. In addition, a cuff was inflated to 60 mm Hg throughout the 30-minute intervention on 1 arm to induce a different SR pattern compared with the noncuffed arm. Before and 5 minutes after each intervention, brachial artery endothelial function was examined using FMD simultaneously in both arms. It has been demonstrated repeatedly that FMD is a largely NO-dependent dilator response in various conduit arteries of humans.[11–15] On 3 additional occasions, endothelium-independent vasodilation was examined in a subgroup (n=6) before and after each intervention. This control experiment was performed to test whether possible changes in FMD relate to acute changes in smooth muscle cell sensitivity to NO. The experimental design, therefore, allowed comparison of different SR patterns under conditions where the mean SRs were matched (interventional comparisons of the noncuffed limb within subjects). In addition, our design allowed comparison of simultaneously derived FMD measures between limbs and within each subject, because cuff inflation altered SR during intervention on the cuffed side.

Experimental Procedures

Vascular function assessments were conducted in a quiet, temperature-controlled environment. Repeated laboratory visits were conducted at the same time of day. Before each test, subjects were requested to fast for 4 hours, abstain from alcohol and caffeine for 12 hours, and avoid exercise for 24 hours.

Assessment of Endothelium-Dependent Vasodilator Function

Before assessment of brachial artery FMD, subjects rested in an upright, seated position for a period of ≥15 minutes to facilitate the baseline measurement of heart rate and blood flow. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap, GE Pro 300V2) placed around the ankle. The hydrostatic column between heart level and the ankle were measured (in centimeters) and converted to millimeters of mercury to calculate the correct blood pressure at heart level.[18]

To examine brachial artery FMD, both arms were extended and positioned at an angle of ≈80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on both forearms immediately distal to the olecranon process to provide a stimulus to forearm ischemia.[19] A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (T3000, Terason) was used to image the brachial arteries in the distal third of the upper arm. When an optimal image was obtained, the probe was held stable, and the ultrasound parameters were set to optimize the longitudinal, B-mode images of the lumen-arterial wall interface. Continuous Doppler velocity assessment was simultaneously obtained using the ultrasound machine and was collected using the lowest possible insonation angle (always <60°), which did not vary during each study. Baseline images were recorded for 1 minute. The forearm cuff was then inflated (>200 mm Hg) for 5 minutes. Diameter and blood flow recordings resumed 30 seconds before cuff deflation and continued for 3 minutes thereafter. These procedures for bilateral and simultaneous FMD assessment were repeated immediately after each intervention, and the same sonographers were involved in each assessment for a given subject.

Assessment of Endothelium-Independent Vasodilator Function Using Glyceryl Trinitrate

In a subset of 6 subjects, we examined brachial artery endothelium-independent vasodilation using glyceryl trinitrate (GTN). Subjects were tested under the same conditions, using the same protocol, set-up, probe placement, and equipment as those described above for the FMD tests. To examine brachial artery GTN%, baseline images were recorded for 1 minute. Subsequently, brachial artery smooth-muscle dilator function was examined after administration of a single spray of sublingual GTN (400 μg), an NO donor. This was followed by a 10-minute recording of the diameter images. GTN assessments were performed before and after each of the 30-minute interventions described below.

Shear Stress Interventions

Immediately after the initial bilateral FMD or GTN assessments, subjects performed a 30-minute intervention. During each intervention, brachial artery diameter and velocity were assessed using a high-resolution ultrasound machine (Aspen, Acuson). We have developed custom-designed software that enables automated edge detection and wall tracking analysis of B-mode images for diameter calculation,[20] combined with synchronized pulse wave velocity waveform detection and consequent real-time (or posthoc) calculation of arterial blood flow, at ≈30 Hz.[21] During each intervention, this system was used to provide real-time feedback of instantaneously calculated blood flow in each arm. This enabled us to adjust exercise workloads such that, in the noncuffed arm, mean blood flows were matched with those recorded during the forearm heating intervention. Subjects performed the cycle and handgrip interventions on separate days, at the same time of day. Images were also recorded onto an S-VHS videocassette recorder (SVO-9500 MDP, Sony) for posthoc analysis using the edge-detection and wall-

tracking software. In addition, heart rate and mean arterial pressure were recorded every 5 minutes throughout the 30-minute intervention using an automated sphygmomanometer around the ankle (Dinamap, GE Pro 300V2).

Forearm Heating

Forearm heating was used as the reference intervention for mean SR elevation, because the heating stimulus was not amenable to rapid adjustment. Forearm heating was performed using the same seated position as the FMD and GTN assessments. Both arms were placed in a separate water bath with 40±1°C water for 30 minutes. Both water baths were maintained at a constant temperature by a thermostatically controlled circulating heater pump.

Recumbent Leg Cycling

Subjects performed the leg cycling exercise on a recumbent bike for 30 minutes at 60 to 70 rpm. Mean blood flow during recumbent leg cycling in the noncuffed arm was matched with that observed during forearm heating in that arm by adjusting the workload, which started at 80 W.

Handgrip Exercise

While seated in the upright position, subjects performed bilateral handgrip exercise using identical dynamometers at a cadence of 30 contractions per minute for 30 minutes (assisted by a metronome). Subjects exercised using an initial weight of 1 to 2 kg, with this intensity subsequently adjusted to match the mean blood flow in the noncuffed arm recorded during forearm heating.

Comparison Between Cuffed and Noncuffed Arms

Both arms were simultaneously exposed to each of the interventions described above. However, a cuff was placed around one arm and inflated to 60 mm Hg during each 30-minute intervention period. Placement of this cuff around the left or right arm was randomized between subjects but always on the same arm for each intervention within subjects. Preliminary pilot studies revealed that 60 mm Hg were sufficient to alter the mean SR and pattern of shear compared with the noncuffed, control arm. Mean SR and the pattern of SR (antegrade versus retrograde) in both the cuffed and noncuffed arms were recorded during each intervention. Preintervention and postintervention FMD or GTN measurements were examined simultaneously in both arms.

Brachial Artery Diameter, Blood Flow, and SR Analysis

Analysis of brachial artery diameters and SR before, during, and after the 3 interventions was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias.[20] See recent articles for detailed descriptions of this analysis approach.[22,23] From this synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) is calculated at 30 Hz. SR (an estimate of shear stress without viscosity) was calculated as 4 times the mean blood velocity/vessel diameter.[24] Reproducibility of diameter measurements using this semiautomated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver coefficient of variation of 6.7%.[20,21] This system was used for posthoc calculation of FMD and GTN responses, as well as during each intervention, to provide real-time feedback for the matching of blood flows across the 3 trials.

Data Analysis

FMD and GTN

FMD and GTN are presented as the absolute (millimeters) and relative (percentage) rises 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.[22] See previous studies for further detail.[22]

In accordance with recent findings,[22,25] we calculated the SR stimulus responsible for endothelium-dependent FMD after cuff deflation. The postdeflation SR data, derived from simultaneously acquired velocity and diameter measures at 30 Hz, were exported to a spreadsheet and the area under the SR curve (AUC_{SR}) calculated for data up to the point of maximal postdeflation diameter (FMD)[22] for each individual using the trapezoid rule.

SR Assessment During the Interventions

The software described above was also used for analysis of SR, derived from simultaneously acquired velocity and diameter measures at 30 Hz, during the 3 interventions in both the cuffed and noncuffed arms. The patterns of SR were also assessed by calculating the area under the curve for all of the antegrade blood flow and shear, as well as the area under the retrograde blood flow and shear recordings.

Statistics

Statistical analyses were performed using SPSS 14.0 software (SPSS Inc). All of the data are reported as mean (SD) unless stated otherwise, whereas statistical significance was assumed at $P<0.05$. Assuming 80% power and an α of 0.05, 9 subjects are required to detect a clinically and physiologically relevant 1.5% difference in the FMD percentage change between conditions. To correct for potential dropout, we have included 10 subjects. One-way ANOVA with repeated measures (with intervention as independent factor) was used to assess differences among the 3 conditions for baseline characteristics and mean SR. A 2-way repeated-measures ANOVA was used to compare the SR patterns between interventions, SR patterns between cuffed and noncuffed arms, preintervention versus postintervention FMDs between conditions, and preintervention versus postintervention FMDs between cuffed and noncuffed arms. Posthoc tests with a least-square difference test were performed when the ANOVA reported a significant main or interaction effect.

Results

Postexercise FMD data from one subject after recumbent leg cycling and from another subject after forearm heating were excluded from the analysis because image quality was deemed inadequate.

Effect of Interventions on SR Responses in the Noncuffed Arm

Baseline characteristics were not significantly different among the 3 exercise interventions (Table 1). A 2-way ANOVA revealed a significant difference in the pattern of SR among the 3 interventions (interaction; $P=0.04$; Figure 1). Posthoc t tests revealed a significantly lower retrograde SR during forearm heating compared with recumbent leg cycling and with handgrip exercise ($P<0.05$; Figure 1). Mean and antegrade SR were similar during forearm heating, recumbent leg cycling, and handgrip exercise (Figure 1).

Effect of SR Interventions on Change in FMD in the Noncuffed Arm

Preintervention FMD percentage was not significantly different among forearm heating, handgrip, and recumbent leg cycling exercise (Figure 2). The interventions did not significantly alter baseline artery diameter (Table 2). Postintervention FMD percentage significantly increased, compared with preintervention, in response to each intervention (ANOVA; $P<0.001$; Figure 2). The 2-way ANOVA revealed no significant interaction effect, indicating a similar increase in FMD percentage for all of the interventions (Figure 2). Indeed, a similar magnitude of increase, expressed in relative (percent-

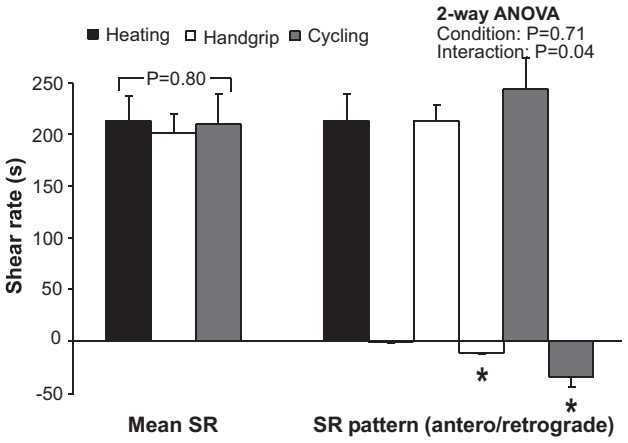

Figure 1. SR (mean, antegrade, and retrograde SR) during heating (■), handgrip (□), and recumbent leg cycling (▩) in healthy young men (n=10) in the noncuffed arm. A 1-way ANOVA was used to examine differences among the 3 interventions for mean SR, whereas a 2-way ANOVA was used to examine the differences in blood flow pattern (antegrade−retrograde SR) among the 3 interventions. *Posthoc was significantly different from forearm heating. Error bars represent SE.

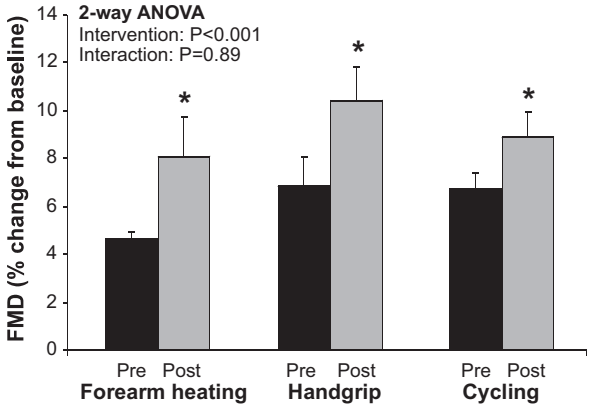

Figure 2. FMD before (black) and after (grey) heating, handgrip, and recumbent leg cycling in healthy young men (n=10) in the noncuffed arm. A 2-way repeated-measures ANOVA was used to examine the impact of the interventions on FMD (main effect) and whether the change in FMD differed among the 3 interventions (interaction). *Posthoc was significant from preintervention. Error bars represent SE.

age; Figure 2) and absolute terms (millimeters; Table 2), was found across all 3 of the interventions. No significant differences were evident in AUC_{SR} between preintervention and postintervention (Table 2), indicating that the eliciting SR stimulus for FMD after cuff deflation was not significantly altered by any of the interventions.

Effect of Cuff Inflation on SR Responses

No differences in preintervention FMD percentage were evident between arms preceding any of the interventions (Figure 3). Mean SR was significantly lower in the cuffed arm, compared with the noncuffed arm, during each intervention (Figure 3; $P<0.05$). For each intervention, antegrade SR in the cuffed arm was significantly lower compared with the noncuffed arm ($P<0.05$), whereas retrograde shear did not significantly differ ($P>0.05$). Therefore, inflating a forearm cuff to 60 mm Hg decreased mean SR principally by altering the antegrade SR for each intervention; it did not significantly modify the retrograde SR pattern.

Effect of Interventions on FMD Between the Cuffed and Noncuffed Arms

Forearm Heating

A 2-way ANOVA revealed no main effect of the intervention on FMD percentage but a significant interaction between both limbs (Figure 3). Although forearm heating significantly increased brachial artery FMD percentage in the noncuffed arm ($P<0.05$), no change was found in the cuffed arm (Figure 3). Also, for the absolute change in brachial artery FMD (millimeters), a significant interaction between both limbs was present (ANOVA; $P<0.05$). The AUC_{SR} was not different between limbs or preintervention versus postintervention (Table 2).

Handgrip

Handgrip exercise increased brachial artery FMD percentage ($P<0.05$) and FMD in millimeters ($P<0.05$) in the noncuffed arm but not in the cuffed arm (Figure 3 and Table 2). Indeed, the 2-way ANOVA revealed a significant interaction effect of handgrip exercise on FMD percentage ($P<0.05$; Figure 3), as well as on FMD in millimeters ($P<0.05$) between the cuffed and noncuffed arms. These effects were present despite a similar AUC_{SR} between pre-exercise and postexercise.

Recumbent Leg Cycling

A significant interaction effect was found between both arms regarding the effect of recumbent leg cycling on FMD percentage ($P<0.005$). An increase in brachial artery FMD percentage in the noncuffed arm was found ($P<0.05$), whereas a decrease in FMD percentage was observed in the cuffed arm ($P<0.05$; Figure 3). Also, when presented as FMD (millimeters), a significant interaction between both arms (2-way ANOVA; $P<0.01$) was evident. The postdeflation AUC_{SR} did not differ between limbs or change after recumbent leg cycling in both arms (Table 2).

Effect of Interventions on GTN on the Cuffed and Noncuffed Arms

Preintervention GTN percentage was not significantly different among forearm heating, handgrip, and recumbent leg cycling exercise in the noncuffed or the cuffed arm (repeated-measures ANOVA: $P=0.72$ and 0.49, respectively). Also, no differences in preintervention GTN percentage were present between the arms under each condition (paired t test: $P=0.33$).

None of the interventions elicited a significant change in brachial artery GTN percentage in either the noncuffed or cuffed arm (Figure 4). No interaction effect of each intervention on GTN was found between the noncuffed or cuffed arm (Figure 4).

Discussion

In the present study we compared the effects of manipulating brachial artery blood flow and shear on FMD in humans. By monitoring real-time blood flow and SR and subsequently modulating exercise intensities, we effectively matched mean SR and blood flow across our 3 interventions, whereas

Table 2. Brachial Artery Baseline Characteristics of Healthy Young Subjects Before (Pre) and After (Post) Each Intervention in the Noncuffed and Cuffed Arms

	Forearm Heating		Handgrip		Recumbent Leg Cycling		
Brachial Artery	Pre	Post	Pre	Post	Pre	Post	ANOVA *P*
Noncuffed arm							
Baseline, mm	4.0±0.8	4.3±0.8	4.1±1.0	3.9±0.6	3.9±0.8	4.0±0.7	0.31
FMD, mm	0.18±0.06	0.30±0.19*	0.26±0.10	0.38±0.12*	0.26±0.10	0.34±0.08*	0.07
FMD, %	4.6±0.9	8.1±5.4*	6.9±3.7	10.4±4.6*	6.7±2.2	8.9±3.2*	0.13
AUC_{SR}	12 679±3138	20 121±13 460	20 121±13 460	18 768±12 313	20 733±16 776	25 470±11 690	0.51
Noncuffed arm							
Baseline, mm	4.1±0.5	4.2±0.5	4.1±0.6	4.0±0.5	3.7±0.6	4.0±0.6	0.12
FMD, mm	0.27±0.11	0.19±0.11	0.29±0.11	0.27±0.10	0.32±0.14	0.25±0.15	0.68
FMD, %	5.8±2.2	4.7±2.2	7.2±3.1	6.7±2.5	8.8±2.7	6.1±3.4	0.11
AUC_{SR}	15 595±5805	16 386±10 861	18 731±13 004	20 427±9454	19 823±11 875	25 117±7397	0.67

Values are mean±SD. The *P* value refers to 1-way repeated-measures ANOVA between the preintervention values. No differences were found between pretest values among the 3 testing days. BAFMD indicates brachial artery FMD.

*Data are significant from pre-exercise at $P<0.05$ (paired *t* test).

antegrade/retrograde patterns significantly differed. The use of bilateral and simultaneous measures also allowed us to manipulate SR using a cuff placed on one arm throughout each intervention, which effectively decreased the magnitude of antegrade flow and shear within subjects. The major findings are as follows: (1) when antegrade SR was increased to a similar extent for 30 minutes using heating, handgrip, or cycle exercise, FMD increased to a similar extent under all 3 of the conditions; (2) decreasing antegrade SR via subdiastolic cuff inflation abolished this increase in FMD; and (3) endothelium-independent NO-mediated vasodilator function was unaffected by a 30-minute period of SR modulation. These results indicate that FMD, a measure of largely NO-dependent, endothelium-dependent vasodilation,[11–15] is modulated by different SR interventions in vivo, primarily because of the differences in the magnitude of antegrade flow and shear.

In a previous study, we observed significant differences in the magnitude of antegrade and retrograde brachial artery flows between handgrip and leg cycling exercise.[17] We speculated that these differences may importantly modulate vascular adaptations in response to exercise training. Indeed, as reviewed recently,[9] studies performed in vitro and in animals suggest that different shear patterns induce different cellular events. Changes in unidirectional shear stress decrease expression of endothelin 1 and vascular cell adhesion molecule 1, whereas oscillatory shear increases endothelin 1[26] and adhesion molecules (vascular cell adhesion molecule 1),[27,28] decreases endothelial NO synthase expression, increases expression of enzymes that produce reactive oxygen species (ie, NADPH oxidase),[29,30] and increases superoxide release.[31] The results of the present study provide some insight into the relative importance of antegrade versus retrograde patterns of blood flow and shear in humans. Differences in mean blood flow and SR between the cuffed and noncuffed arms, primarily because of differences in the antegrade SR, resulted in different impacts on FMD, whereas the endothelium-independent responses to GTN were unaffected. This suggests that changes in antegrade blood flow and SR provide an important stimulus to acute enhancement of endothelial function in vivo. Conversely, significant differences in the magnitude of retrograde blood flow between the interventions in the noncuffed arm did not significantly influence the change in FMD. This suggests that, under the experimental conditions of the present study, retrograde blood flow and shear did not markedly influence endothelial function. This should not infer that changes in retrograde flow and shear are not important, because we recently found a dose-response relationship between increases in retrograde shear and impairment in FMD responses.[32] Taken together, these data suggest that the magnitude of antegrade flow and shear provide an important stimulus to acutely increase endothelial function in humans and that increases in antegrade shear may also prevent any impairment in endothelial function associated with unopposed increases in retrograde flow and shear.

The implications of this study relate to the impact of exercise and physical conditioning on vascular adaptations. Although exercise training and physical activity are associated with substantial cardiovascular benefit, recent evidence suggests that exercise-mediated changes in traditional and novel risk factors fail to fully account for this benefit.[6,33] Exercise training can enhance endothelial function and induce endothelium-dependent arterial remodeling in humans.[34] Therefore, it has been suggested that the direct antiatherogenic effects of exercise on the vasculature, mediated through changes in shear stress and possibly perfusion pressure associated with each exercise bout,[6,9,34] may account for the unexplained beneficial impact of training on cardiovascular risk.[6] The present study indicates that changes in the magnitude of antegrade blood flow and SR, which are typically associated with exercise, are indeed associated with an acute improvement in brachial artery endothelial function. Future studies involving exercise training are necessary to further establish the importance of blood flow and SR to induce vascular adaptations and the consequent cardiovascular benefit.

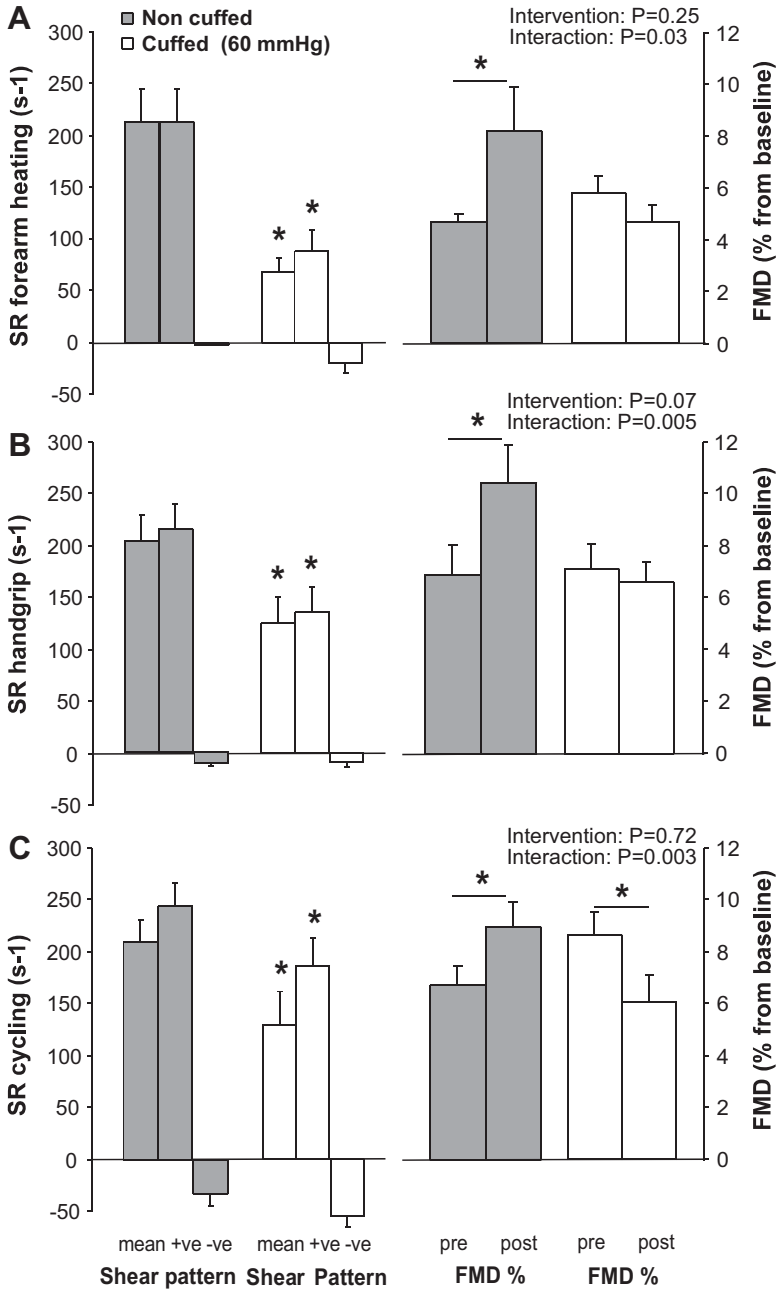

Figure 3. Mean SR and SR pattern during the intervention (+ve, antegrade SR; −ve, retrograde SR) and FMD before and after the intervention are presented for the noncuffed (▨) and cuffed arms (□). Data are presented for forearm heating (A), handgrip (B), and recumbent leg cycling (C) in healthy young men (n=10). Mean and pattern of shear between the noncuffed and cuffed arms during each intervention are tested with a paired *t* test (*$P<0.05$). A 2-way ANOVA is used to examine whether cuff placement influenced the change in FMD on the 30-minute intervention (*Posthoc was significant between preintervention and postintervention). Error bars represent SE.

Many studies indicate that vascular adaptations to exercise training are not only localized to the active muscle bed but may also be evident in arteries not directly feeding the active skeletal tissue.[6] For example, changes in brachial artery endothelial function are evident after lower limb exercise training.[34,35] In the present study, cycle ergometer exercise had similar effects on brachial artery FMD as those evident in response to forearm handgrip exercise. This suggests that sufficient increase in antegrade blood flow and SR, which occurs as a consequence of hemodynamic responses associated with large muscle mass exercise, can enhance endothelial function in vascular beds feeding inactive skeletal muscle.

Several limitations of the present study are germane. Although the sample size was relatively modest, it is in keeping with physiological studies of this nature, and the results were consistent between and within individuals. Recruiting a larger number of subjects would not have importantly changed our findings. We studied men to avoid the established cyclic effects of sex hormones on vascular function in women. Similarly, the subjects who we recruited were young and healthy with normal endothelial function. One limitation relates to the fact that we did not perform blockade experiments to prove that our FMD changes were endothelium or NO dependent. Nonetheless, FMD has consistently been demonstrated as an NO- and endothelium-dependent stimulus in human conduit arteries.[11–15] We suggest that the method presented here, involving clamping of mean SR by matching these between conditions, and simultaneous assessment of FMD bilaterally with unilateral manipulation may provide a useful method for future investigation of the impact

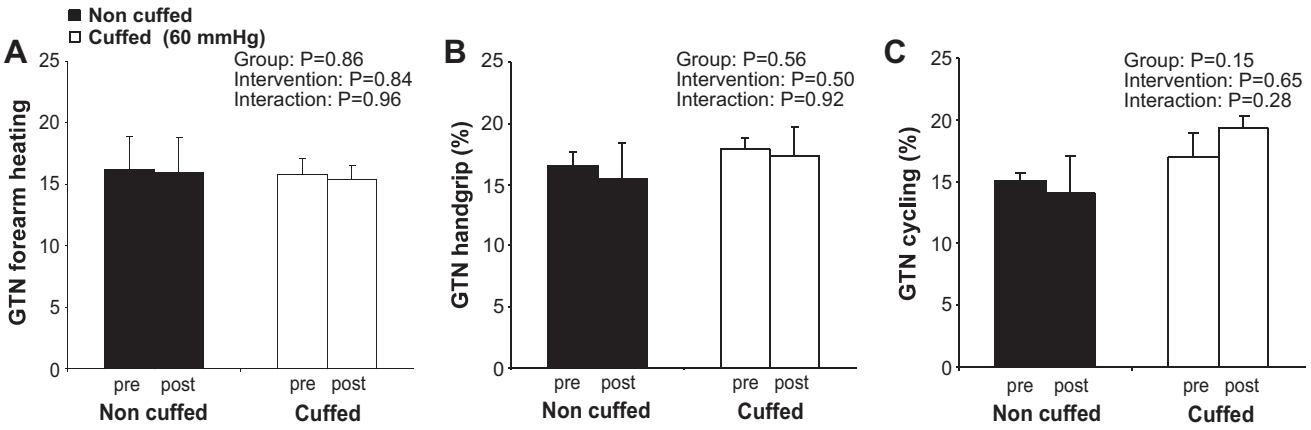

Figure 4. Endothelium-independent vasodilation examined using GTN before and after the intervention is presented for the noncuffed (■) and cuffed arms (□). Data are presented for forearm heating (A), handgrip (B), and recumbent leg cycling (C) in healthy young men (n=6). A 2-way ANOVA did not find an effect of the intervention of the GTN response in the noncuffed or cuffed arm. Error bars represent SE.

of various interventions or conditions on vascular function, because it minimizes the impact of between-subject variability and repeated-assessment effects.

Perspectives

In this study, we manipulated the antegrade versus retrograde patterns of blood flow and SR using 3 distinct stimuli. When the magnitude of increase in antegrade SR was similar across these interventions, we observed similar improvements in FMD. Conversely, when the rise in antegrade blood flow and SR was attenuated within subjects using unilateral cuff inflation, the increase in FMD was abolished. These data suggest that differences in the antegrade/retrograde pattern of SR, and in particular changes in antegrade shear and blood flow, have important modulating impacts on endothelial function in vivo. As exercise is typically associated with elevations in antegrade SR, this finding may have relevance for the impact of different exercise interventions on vascular adaptations in humans.

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Disclosures

None.

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