ORIGINAL ARTICLE

Effect of external compression on femoral retrograde shear and microvascular oxygenation in exercise trained and recreationally active young men

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Abstract

Introduction Retrograde shear causes endothelial damage and is pro-atherogenic. The purpose of our study was to examine the impact of vascular remodeling from habitual exercise training on acute changes in retrograde shear and microvascular oxygenation (SMO_2) induced via 30 min of external compression.

Methods Participants included 11 exercise trained (ET) men (Division I track athletes; age 20 ± 3 years) and 18 recreationally active (RA) men (age 23 ± 5 years). Near-infrared spectroscopy (NIRS) was used to measure vastus medialis SMO_2. Doppler-ultrasound was used to assess SFA intima-media thickness, diameter and flow velocity to derive retrograde shear. Vascular measures were made at baseline (BASELINE), during a sham condition (calf compression to 5 mmHg, SHAM) and during the experimental condition (calf compression to 60 mmHg, EXP).

Results Compared to RA, ET had larger SFA diameters (0.66 ± 0.06 vs 0.58 ± 0.06 cm, $p < 0.05$) and lower SFA IMT (0.33 ± 0.03 vs 0.36 ± 0.07 mm, $p < 0.05$). Retrograde shear increased similarly in both groups during EXP ($p < 0.05$) but ET men had lower overall retrograde shear during the conditions (BASELINE 75.8 ± 26.8 vs EXP 88.2 ± 16.9 s^{-1}) compared to RA men (BASELINE 84.4 ± 23.3 vs EXP 106.4 ± 19.6 s^{-1} $p < 0.05$). There was a similar increase in SMO_2 from BASELINE to SHAM (ET $+ 8.1 \pm 4.8$ vs RA $+ 6.4 \pm 9.7$%) and BASELINE to EXP (ET $+ 8.7 \pm 6.4$ vs RA $+ 7.1 \pm 9.0$%) in both groups.

Conclusion Beneficial vascular remodeling in ET men is associated with lower retrograde shear during external compression. Acute increases in retrograde shear with external compression do not detrimentally impact microvascular oxygenation.

Keywords Retrograde shear · Vascular · Arterial stiffness · Exercise training

Abbreviations
BMI	Body mass index
DBP	Diastolic blood pressure
ET	Exercise-trained
HR	Heart rate
IPAQ	International Physical Activity Questionnaire
IMT	Intima-media thickness
SMO_2	Muscle oxygen saturation
NIRS	Near-infrared spectroscopy
PWV	Pulse wave velocity
RA	Recreationally active
SV	Stroke volume
SFA	Superficial femoral artery
SBP	Systolic blood pressure

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Introduction

Blood flow is triphasic in the peripheral conduit arteries and is characterized as having a systolic antegrade (forward) component, an early diastolic retrograde (backward) component and a late diastolic antegrade component. Antegrade shear is anti-atherogenic and promotes optimal vascular endothelial function. Contrastingly, excessive retrograde shear can damage the vascular endothelium and promote a pro-atherogenic phenotype (Schreuder et al. 2015). Peripheral shear patterns are dynamic and change with posture (Padilla et al. 2009), temperature (Tinken et al. 2009), activity/inactivity/exercise (Johnson et al. 2012; Restaino et al. 2016) and any other perturbation that effect sympathetic/α-adrenergic tone (Casey et al. 2012; Thijssen et al. 2014).

With the multitude of stimuli that can alter peripheral shear patterns, it has become increasingly important to understand the potential vascular consequences of such changes in shear patterns.

Mild external compression is used experimentally as a means to manipulate retrograde shear and examine the effects on vascular function. Acute exposure (~ 30 min) to increased retrograde shear from cuff compression leads to regional conduit artery endothelial dysfunction in both the upper and lower limbs (Schreuder et al. 2015; Zepetnek et al. 2014, 2015). The effects of retrograde shear on the microvasculature remain less explored. A recent study by Ramos Gonzalez et al. found that lower limb microvascular reactivity is preserved following acute increases in conduit vessel retrograde shear (Ramos Gonzalez et al. 2018), suggesting that the vascular response to retrograde shear may differ between larger conduit vessels and smaller resistance vessels. Given the importance of the microcirculation for regulating numerous biological functions, such as tissue perfusion/oxygenation, understanding microvascular function during epochs of increased retrograde shear requires further scrutiny.

Habitual exercise training improves peripheral vascular structure and function through direct hemodynamic changes and favorable vascular remodeling (Green et al. 2012). Notable peripheral vascular changes include increased artery diameter, lower vessel wall thickness and enhanced conduit vessel and microvascular reactivity (Green et al. 2012). The effects of habitual exercise training on shear patterns remains less studied. We have previously reported no differences in superficial femoral artery (SFA) retrograde shear in young exercise-trained women compared to sedentary women (Augustine et al. 2016). Similarly, Casey et al. noted no differences in common femoral artery retrograde shear in older exercise-trained women compared to older sedentary women (Casey et al. 2016). Interestingly, older exercise trained men have lower femoral retrograde shear than older sedentary men (Casey et al. 2016). Whether younger exercise-trained men have more favorable shear patterns compared with less active men has not been specifically explored. Moreover, whether habitually exercise-trained men have a differential microvascular response during acute increases in retrograde shear remains unknown.

The purposes of this study was to examine SFA shear and microvascular oxygenation in exercise-trained (ET) men versus recreationally active (RA) controls during lower-limb compression. Regional SFA hemodynamics were assessed using a combination of Doppler-ultrasound and near infrared spectroscopy (NIRS) as each offers unique insight into the vascular landscape (Ives et al. 2014; Jones et al. 2016). It was hypothesized that: (1) habitually ET men would have lower retrograde shear and higher microvascular oxygenation during compression

of the lower limb owing to more optimal vascular remodeling (larger SFA diameter and lower IMT); and (2) acute increases in retrograde shear during external compression would not affect microvascular oxygenation.

Methods

Participants

Twelve habitually ET men (Division I track athletes engaging in middle-distance and long-distance events; 20 ± 3 years of age, body mass index 21 ± 2 kg m^{-2}) and 18 RA controls (23 ± 5 years of age, body mass index 23 ± 2 kg m^{-2}) volunteered to participate in the study. Exclusion criteria for participants consisted of self-reported (from a health history questionnaire) hypertension, smoking, diabetes mellitus, hyperlipidemia, pulmonary disease, renal disease, neurological disease, peripheral artery disease, BMI ≥ 30 kg m^{-2} or recent injury to the lower extremities. One ET participant was excluded owing to recent surgery (within 1 week) stemming from a lower extremity injury. Controls were operationally defined as RA if meeting Physical Activity Guidelines recommendation of 500–1000 MET-min per week of total energy expenditure and were not participating in any Division I or club sports. Physical activity levels were determined via self-report using the International Physical Activity Questionnaire (IPAQ). Following approval by the Institutional Review Board for Research at Syracuse University, written informed consent was obtained from each participant prior to testing.

Experimental design

Participants reported to the research lab for one data collection session. On the testing day, participants were asked to fast ≥ 3 h prior to participation, and to refrain from vigorous exercise, alcohol, and caffeine consumption for 24 h prior to testing. All tests were conducted in a quiet, dimly lit, temperature-controlled laboratory. Upon arrival, participants completed the consent process, completed health history questionnaires along with the International Physical Activity Questionnaire, and underwent anthropometric and body composition measurement. Stature was measured with a stadiometer and body mass with an electronic scale. Body fat% was assessed via air displacement plethysmography. Following anthropometric assessment, participants performed a seated leg extension against no resistance enabling researchers to identify the vastus medialis. A near infrared spectroscopy (NIRS) device (MOXY, Fortiori Design LLC) was attached to the vastus medialis of the participant. Participants then laid supine for a 10-min habituation period. A brachial blood pressure cuff was wrapped around the
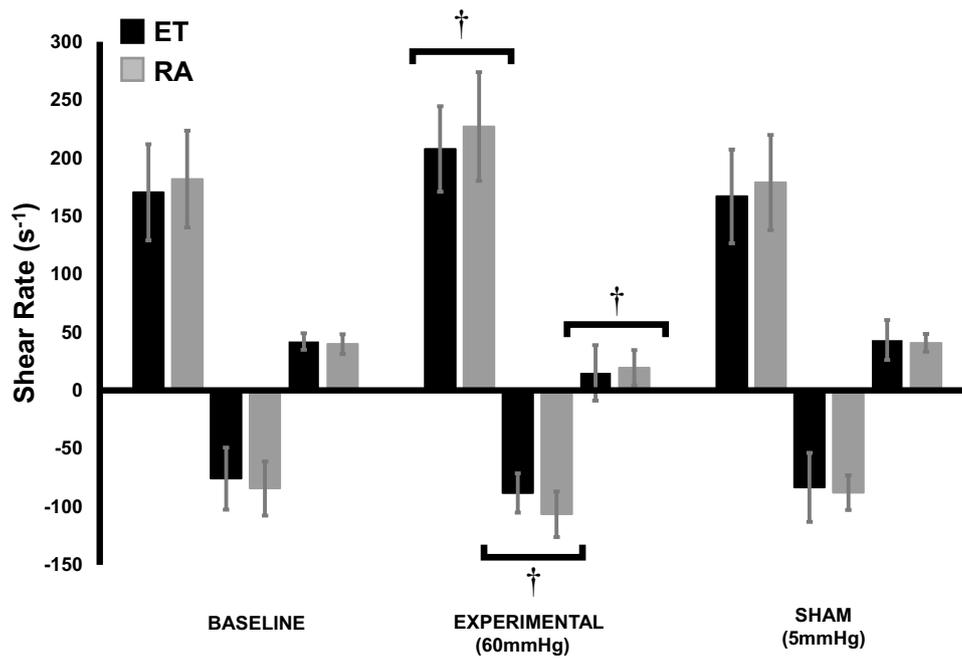
Fig. 1 Superficial femoral artery shear at BASELINE, during SHAM (5 mmHg compression) and during EXP (60 mmHg compression) conditions. Black bars represent exercise trained (ET) men while grey bars represent recreationally active (RA) men. Positive values indicate both primary (systolic) and secondary (diastolic) antegrade shear while negative values indicate retrograde shear. Systolic ante- grade shear: Group effect, $p=0.34$; Condition effect, $p<0.001$; Group×Condition interaction, $p=0.75$. Retrograde shear: Group effect, $p=0.02$; Condition effect, $p=0.01$; Group×Condition interac- tion, $p=0.19$. Diastolic antegrade shear: Group effect, $p=0.97$; Con- dition effect, $p<0.001$; Group×Condition interaction, $p=0.74$. [†]Sig- nificantly different from BASELINE ($p<0.05$)

Fig. 2 Absolute values for muscle oxygenation (SMO_2) at baseline, during SHAM (5 mmHg compression) and during EXP (60 mmHg com- pression) conditions. Black bars represent exercise trained (ET) men while grey bars represent recreationally active (RA) men. Positive values indicate both primary (systolic) and second- ary (diastolic) antegrade shear while negative values indicate retrograde shear. Group effect, $p=0.045$; Condition effect, $p<0.001$; Group×Condition interaction, $p=0.42$. [†]Signifi- cantly different from BASE- LINE ($p<0.05$)
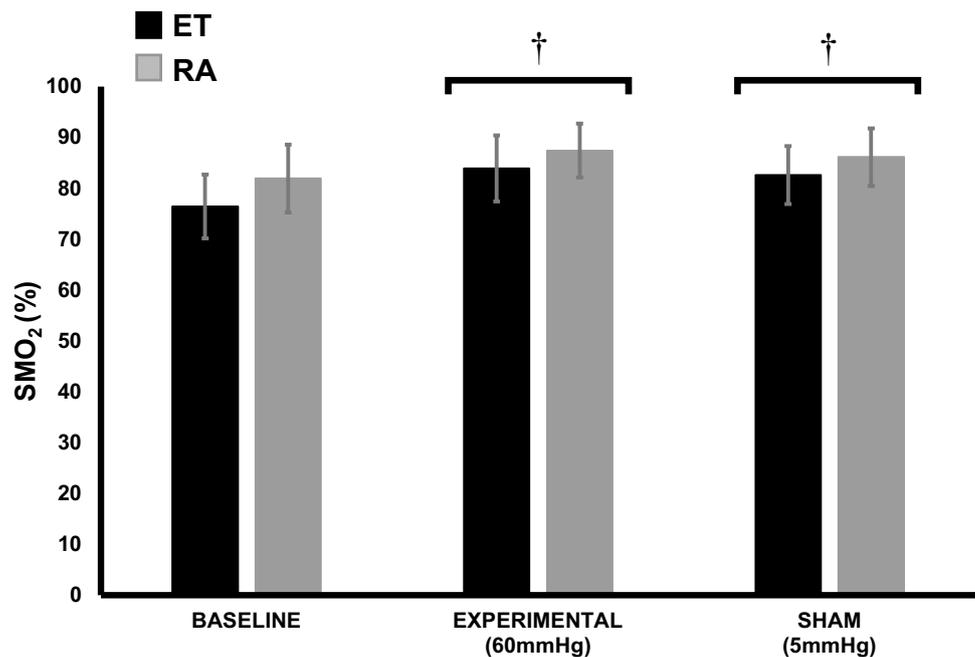
participant's upper arm and ECG electrodes placed under the clavicle and on the lower ribs in a modified CM5 configura- tion. A tourniquet-style pneumatic cuff was wrapped around

the lower left leg (calf) at the area of greatest circumference. Participants rested in the supine position for an additional 20 min before the start of data acquisition.

Study conditions included a rest/baseline condition (BASELINE), compression of the lower limb to 5 mmHg as a sham condition (SHAM), and compression of the lower limb to 60 mmHg as the experimental condition (EXP). Compression pressure was selected based on previous work from Schreuder et al. (2014). Compression conditions were completed in a randomized order and each compression condition was carried out for 30 min as previous research has shown this amount of time sufficient to induce acute endothelial damage (Thijssen et al. 2009). Each compression condition was followed by a 5-min recovery period. Brachial blood pressure measurements along with simultaneous assessment of superficial femoral artery (SFA) flow velocity and diameter via Doppler-ultrasound were acquired during BASELINE, during each compression condition (SHAM and EXP) and the recovery period (recovery data not shown). Two steady state blood pressures were taken during BASELINE and averaged for subsequent data analysis. Brachial blood pressure measures and Doppler-ultrasound measures were made once during each compression condition at the midway point (15 min) to confirm the stimulus (i.e., change in shear patterns). Brachial pressure waveforms captured at each time point were further used to derive measures of central hemodynamics (aortic stiffness, augmentation index, central blood pressure, cardiac output, stroke volume, total peripheral resistance). NIRS data were continuously monitored throughout rest and each compression period.

NIRS

A light (48 g) and compact ($61 \times 44 \times 21$ mm) device (Muscle Oxygen Monitor [MOXY], Fortiori Design LLC) was secured to the vastus medialis using a customized Velcro bandage that blocked out ambient light sources and used to continuously capture data at baseline, throughout each condition and the intercalated recovery periods. This valid and reliable device is composed of a single LED emitter optode that emits near infrared light, and two NIRS detector optodes placed at 12.5 and 25.0 mm from the light source (Crum

et al. 2017; McManus et al. 2018). The device provides a non-invasive measurement of microvascular oxygenation that uses the light absorption at wavelengths of 630–830 nm into the tissue to quantify total tissue hemo($+$ myo)globin and muscle oxygen (SMO_2) in the vastus medialis using the following formula:

$$\left(\frac{\text{Oxygenated hemoglobin} + \text{myoglobin}}{\text{total amount of hemoglobin} + \text{myoglobin}} \right) \times 100 = SMO_2.$$

Due to similar absorption characteristics by hemoglobin and myoglobin, values recorded by the MOXY device were used to calculate percent change of SMO_2 for each experimental condition. Data were obtained from the device's internal memory at a rate of 2 Hz using MOXY software. These data were averaged into 5-min bins for offline analysis. The last 5 min of each condition was used for subsequent analyses. Data are presented as both absolute and relative (change from baseline) values.

SFA measures

A 13.0 MHz linear array probe was used to obtain images and flow velocity waveforms of the left SFA via Doppler-ultrasound (ProSound α7, Aloka, Tokyo, Japan) as we have previously described (Heffernan et al. 2013). Three images were obtained from each condition (BASELINE, SHAM, and EXP, as well as the subsequent recovery conditions). These were obtained during the end of the T-wave and peak R-wave of the ECG as timing markers for cardiac cycle phase. Diameter was measured from a longitudinal image from the near wall to the far wall lumen of the SFA and gated to the ECG. Minimum diameter was used for calculation of shear rates (described below). The boundary between the intima and media (also during R-wave peak) was measured using digital calipers as the intima-media thickness (IMT). To adjust for potential group differences in lumen size, the IMT-to-lumen ratio was calculated (IMT/lumen).

Intra-rater and inter-rater reliability for IMT analysis in our lab (calculated on 2 separate days on ten randomly selected images) is presented below. Data shown are the coefficient of variation and the intra-class correlation coefficient with associated 95% confidence intervals.

Researcher 1 intra-rater reliability: $3.6 \pm 6.6\%$[ICC: $0.748(0.046 - 0.936)$],

Researcher 2 intra-rater reliability: $6.8 \pm 8.9\%$[ICC: $0.545(- 0.720 - 0.885)$],

Inter-rater reliability: $5.5 \pm 4.7\%$[ICC: $0.773(0.144 - 0.943)$].

Flow velocity waveforms were measured using an insonation angle of 60° for all measures. At least four waveforms were averaged to obtain a representative average waveform. This was done using eTracking software. Systolic antegrade, diastolic retrograde, and diastolic antegrade, and mean blood velocity (V_m) were measured with Doppler ultrasound. V_m was computed with the following formula: $V_m = \int V(t)\mathrm{d}t/\mathrm{FT}$. $\int V(t)\mathrm{d}t$ is the velocity–time integral of the velocity waveform and FT is flow time. Shear rate was calculated using the following formula: Shear rate $= 4 \times (V_m/\text{diameter})$ (Casey et al. 2012; Padilla et al. 2009; Heffernan et al. 2013). The resistance index (RI) was calculated as $V_s - V_d/V_s$ where V_s is systolic velocity and V_d is diastolic velocity.

Blood pressure and systemic hemodynamics

Brachial pulse waveforms were captured using an automated oscillometric cuff (Mobil-O-graph®, IEM). In addition to providing measures of brachial systolic and diastolic pressure, several additional hemodynamic parameters were derived from the pulse waveform using the ARC-Solver method. This method uses a modified 3-element Windkessel model and transmission line theory to estimate an aortic flow velocity waveform. With the combination of the central pressure and flow waveform several static hemodynamic outputs can be estimated including: central systolic blood pressure (cSBP), central diastolic blood pressure (cDBP), aortic pulse wave velocity (PWV), aortic augmentation index (AIx), reflection magnitude (RM), stroke volume (SV), heart rate (HR), and total peripheral resistance (TPR) (Feistritzer et al. 2015; Jones et al. 2000; Weber et al. 2011). These measures were acquired to confirm that our regional manipulation did not have any systemic hemodynamic effects that might affect interpretation of findings.

Statistical analyses

All statistical analyses were conducted using IBM SPSS version 24 (SPSS Inc., Chicago, IL). All data are reported as mean ± standard deviation. Statistical significance was established priori at *p < 0.05*. A two by three repeated measures analysis of variance was used to test for group effects (ET vs RA), condition effects (BASELINE, SHAM, EXP), and group-by-condition interactions. Repeated measures analysis of variance were conducted with order of condition entered as a covariate to test for possible order effects. A Bonferroni correction was made to adjust for multiple comparisons.

Table 1 Participant characteristics

	Exercise-trained ($n=11$)	Recreationally active ($n=18$)	p value
Age (years)	20 ± 3	23 ± 5	0.22
Height (m)	1.89 ± 0.06	1.81 ± 0.07	0.64
Weight (kg)	76.0 ± 9.5	74.5 ± 7.9	0.12
Body mass index (kg m^{-2})	21.5 ± 1.9	23.5 ± 1.7	0.01
Body fat (%)	6 ± 3	14 ± 6	0.001
Resting heart rate (bpm)	52 ± 10	61 ± 12	0.03
Resting systolic pressure (mmHg)	123 ± 6	127 ± 8	0.10
Resting diastolic pressure (mmHg)	71 ± 8	74 ± 7	0.22
Vigorous activity (min/week)	252 ± 121	158 ± 113	0.04
Moderate Activity (min/week)	214 ± 149	77 ± 94	0.006
Walking (min/week)	244 ± 144	299 ± 417	0.67
Sitting time (min/week)	2095 ± 911	3028 ± 1293	0.04

Univariate relationships of interest were assessed using Spearman correlation coefficients.

Results

Descriptive characteristics

Participant characteristics are presented in Table 1. Groups were similar in age, stature and body mass ($p > 0.05$). ET men had lower body fat compared to RA men ($p < 0.05$).

Peripheral vascular-hemodynamic and microvascular parameters

SFA parameters are presented in Table 2. ET men had smaller SFA IMT:lumen ratios compared to RA men ($p < 0.05$) at BASELINE owing to larger SFA diameters ($p < 0.05$) and smaller IMT ($p < 0.05$). There were no changes in SFA IMT:lumen ratio across conditions ($p > 0.05$). Significant condition effects were noted for both systolic and diastolic antegrade shear (Fig. 1, $p < 0.05$). Both groups of men experienced similar increases in systolic antegrade shear and similar reductions in diastolic antegrade shear during the EXP condition ($p < 0.05$). A group effect and condition effect were detected for retrograde shear (Fig. 1, $p < 0.05$). ET men had lower retrograde shear across all conditions compared to RA men ($p < 0.05$). Both groups of men experienced similar increases in retrograde shear

Table 2 Peripheral vascular-hemodynamic measures

	Baseline	Experimental (60 mmHg)	Sham (5 mmHg)	Group effect	Condition effect	Group × Condition interaction
Diastolic diameter (cm)						
Exercise-trained	0.66 ± 0.06	0.66 ± 0.06	0.66 ± 0.04	0.02	0.22	0.15
Recreationally active	0.58 ± 0.06	0.59 ± 0.06	0.59 ± 0.06			
IMT (mm)						
Exercise-trained	0.33 ± 0.03	0.33 ± 0.04	0.33 ± 0.03	0.04	0.47	0.49
Recreationally active	0.36 ± 0.07	0.37 ± 0.05	0.36 ± 0.05			
IMT:lumen ratio						
Exercise-trained	0.053 ± 0.01	0.052 ± 0.01	0.057 ± 0.02	0.04	0.86	0.15
Recreationally active	0.064 ± 0.01	0.065 ± 0.01	0.061 ± 0.01			
Mean shear rate (s^{-1})						
Exercise-trained	54.8 ± 34.2	29.2 ± 23.8[†]	53.1 ± 37.4	0.75	0.001	0.85
Recreationally active	57.1 ± 22.7	34.5 ± 13.0[†]	53.9 ± 23.2			
Resistance index						
Exercise-trained	1.12 ± 0.06	1.36 ± 0.21[†]	1.16 ± 0.06	0.12	0.001	0.72
Recreationally active	1.10 ± 0.06	1.29 ± 0.16[†]	1.13 ± 0.04			

[†]Different from Baseline ($p < 0.05$)

during the EXP condition (p > 0.05), thus no group-by-condition interaction was detected. Significant condition effects were also noted for SFA RI ($p < 0.05$). Both groups of men experienced similar increases in RI during EXP ($p < 0.05$). A significant condition effect was detected for SMO_2 (Fig. 2, $p < 0.05$). There was a similar change in SMO_2 from BASELINE to EXP (ET $+ 8.7 \pm 6.4$ vs RA $+ 7.1 \pm 9.0\%$) and BASELINE to SHAM in both groups (ET $+ 8.1 \pm 4.8$ vs RA $+ 6.4 \pm 9.7\%$). No order effects were detected for aforementioned outcomes ($p > 0.05$).

Systemic vascular-hemodynamic parameters

There was no change in systemic vascular-hemodynamic parameter across conditions (Table 3, $p > 0.05$) suggesting that the lower limb compression did not impact systemic hemodynamics. Group effects were detected for SV, cSBP, PWV, AIx and HR ($p < 0.05$) where ET men had greater SV but lower cSBP, PWV, AIx and HR compared to RA men (Table 3).

Correlations between SFA shear patterns and microvascular oxygenation

Correlates of retrograde shear at BASELINE included IMT:lumen ratio ($r = 0.54$, $p < 0.05$). Correlates of change in retrograde shear during EXP included: aortic PWV ($r = 0.48$, $p < 0.05$) and RM ($r = 0.34$, $p < 0.05$). Neither BASELINE retrograde shear nor EXP retrograde shear were associated

with TPR or RI ($p > 0.05$). SMO_2 at BASELINE was associated with mean shear ($r = 0.37$, $p < 0.05$), systolic antegrade shear ($r = 0.35$, $p < 0.05$) and diastolic antegrade shear ($r = 0.45$, $p < 0.05$) but not retrograde shear ($r = 0.22$, $p > 0.05$). During the EXP condition, SMO_2 was not associated with any measure of shear ($p > 0.05$).

Discussion

The purpose of this study was to determine whether regional vascular structural remodeling from habitual exercise training alters the blood flow response (retrograde shear and microvascular oxygenation) to lower limb compression in young, healthy men. ET men had smaller SFA IMT:lumen ratio. This favorable vascular remodeling likely contributed to ET men having lower retrograde shear at rest and during external compression compared to RA men. Both the EXP and SHAM conditions resulted in a similar increase in microvascular oxygenation in both ET and RA men. Given that increases in microvascular oxygenation occurred concomitantly with increases in retrograde shear, we interpret findings to suggest that acute increases in retrograde shear from lower limb cuff compression do not detrimentally impact microvascular oxygenation in young, healthy men.

Consistent with previous studies (Heffernan et al. 2013; Schreuder et al. 2014, 2015; Totosy de Zepetnek et al. 2014), there were significant increases in retrograde shear during the EXP condition in comparison to the SHAM condition in both ET and RA men. Interestingly, ET men maintained

Table 3 Systemic vascular-hemodynamic measures

	Baseline	Experimental (60 mmHg)	Sham (5 mmHg)	Group effect	Condition effect	Group × condition interaction
bSystolic blood pressure (mmHg)						
Exercise-trained	123 ± 6	120 ± 7	122 ± 7	0.13	0.10	0.99
Recreationally active	127 ± 8	125 ± 10	126 ± 10			
bDiastolic blood pressure (mmHg)						
Exercise-trained	71 ± 8	67 ± 7	69 ± 8	0.11	0.02	0.59
Recreationally active	74 ± 7	72 ± 8	74 ± 8			
cSystolic blood pressure (mmHg)						
Exercise-trained	108 ± 9	105 ± 7	107 ± 7	0.008	0.09	0.90
Recreationally active	116 ± 8	114 ± 10	115 ± 10			
cDiastolic blood pressure (mmHg)						
Exercise-trained	72 ± 7	68 ± 8	71 ± 8	0.14	0.02	0.47
Recreationally active	75 ± 8	74 ± 8	75 ± 8			
Pulse wave velocity (m s^{-1})						
Exercise-trained	5.1 ± 0.4	5.0 ± 0.3	5.0 ± 0.4	0.005	0.13	0.98
Recreationally active	5.4 ± 0.3	5.4 ± 0.4	5.4 ± 0.4			
Augmentation index @75 (%)						
Exercise-trained	1 ± 11	3 ± 13	3 ± 13	0.04	0.99	0.54
Recreationally active	11 ± 11	8 ± 12	8 ± 16			
Reflection magnitude (%)						
Exercise-trained	50 ± 7	48 ± 6	52 ± 8	0.001	0.39	0.21
Recreationally active	57 ± 5	58 ± 9	58 ± 8			
Stroke volume (mL)						
Exercise-trained	107.9 ± 10.0	109.6 ± 36.5	103.4 ± 30.4	0.05	0.69	0.58
Recreationally active	90.7 ± 14.3	92.9 ± 20.2	92.2 ± 19.5			
Total peripheral resistance (aU)						
Exercise-trained	1.08 ± 0.17	1.07 ± 0.2	1.14 ± 0.22	0.85	0.57	0.77
Recreationally active	1.12 ± 0.17	1.09 ± 0.17	1.11 ± 0.24			
Heart rate (bpm)						
Exercise-trained	52 ± 8	51 ± 10	51 ± 9	0.05	0.06	0.49
Recreationally active	61 ± 12	59 ± 13	59 ± 10			

b brachial, *c* central, *mmHg* millimeters of mercury, *m/s* meters per second, *mL* millilitres, *aU* arbitrary units, *bpm* beats per minute

lower retrograde shear across all conditions compared to RA men, having a slightly attenuated increase with compression. Our results support previous work from Casey et al. (2016) who found that older exercise-trained men had lower retrograde shear in the common femoral artery (Casey et al. 2016). The origins of peripheral retrograde shear are not fully known but likely related to both regional and downstream vascular-hemodynamic factors (Heffernan et al. 2013). Habitual endurance exercise training results in conduit vessel remodeling (larger diameter and lower wall thickness) (Dinenno et al. 2001) and this was seen in our ET men. Regional vascular remodeling may contribute to lower overall mean shear and retrograde shear as larger vessels with lower IMT will experience less shear. Indeed, in our study IMT:lumen was associated with mean and retrograde shear. However, correlation does not imply causation and an alternative interpretation of these associations is that shear patterns may drive vascular adaptations to exercise training (Tanahashi et al. 2017).

Retrograde shear may be influenced by additional central and peripheral vascular factors. Increases in peripheral vascular resistance may increase retrograde shear via a "bottleneck" effect downstream resulting in a blunted diastolic flow run-off and translocation of antegrade flow in diastole retrogradely (Halliwill and Minson 2010). However, in our study there was no association between retrograde shear and measures of peripheral vascular resistance. Moreover, ET and RA men had similar TPR and SFA RI arguing against downstream vascular resistance as the origin of differences in retrograde shear. Peripheral shear patterns may also be

influenced by large artery compliance and pressure from wave reflections (Breton-Romero et al. 2016; Hashimoto and Ito 2010). Pressure from wave reflections may attenuate antegrade flow while augmenting retrograde flow (Heffernan et al. 2013). In the present study, there were associations between retrograde shear and central artery stiffness and pressure from wave reflections. Moreover, ET individuals had lower aortic stiffness and pressure from wave reflections. Finally, habitual exercise training is associated with lower vascular sympathetic tone which in turn favorably effects vascular structure and shear patterns (Dinenno et al. 2000; Moreau et al. 2006). In support of this, we noted that ET men had lower IMT which may be influenced by both vascular structure and vascular tone (Thijssen et al. 2011).

While previous studies have shown that acute exposure of conduit arteries to retrograde shear from external limb compression for 30 min reduces conduit vessel endothelial function, our findings suggest that this manipulation does not have a detrimental effect on microvascular oxygenation. Both compression conditions resulted in similar, albeit small, increases in SMO_2 and this occurred concomitant with disparate changes in retrograde shear (i.e. an increase during the EXP condition and no change during the SHAM condition). Moreover, microvascular oxygenation was not associated with retrograde shear produced by external compression. Our findings are consistent with recent work from Ramos-Gonzalez et al. that reported acute exposure to retrograde shear in the lower limb induced via external compression does not adversely impact NIRS-derived measures of microvascular reactivity in young, healthy men and women (Ramos Gonzalez et al. 2018).

Microvascular oxygenation may be influenced by other components of the shear profile, namely antegrade shear, and thus overall mean shear (i.e. total inflow). Indeed, we noted an association between SMO_2 and antegrade shear at BASELINE. We chose to present antegrade shear as separate components in keeping with the natural tri-phasic SFA flow velocity profile. Each component of antegrade shear reflects distinct physiology. Primary antegrade shear occurring in systole is likely due to a combination of factors including cardiac contractility, vessel diameter and thickness, and vessel compliance (i.e. vascular input impedance). Blood flow temporarily stored in the compliant vessel wall during systole is imparted back to the systemic circulation as antegrade flow during diastole. Thus, secondary antegrade shear occurring in diastole is related to regional vascular compliance (Windkessel effect or reservoir function) and downstream vascular resistance affecting diastolic run-off. In our study, both components of antegrade shear were associated with microvascular oxygenation at BASELINE. Overall, retrograde shear may be an important determinant of endothelial dysfunction and vascular reactivity, while antegrade and

mean shear may be determinants of microvascular oxygenation in the resting state.

Compression of the lower limb to 60 mmHg (EXP condition) increased systolic antegrade shear in the SFA while reducing diastolic antegrade shear. Changes in antegrade shear during external compression are likely related to regional increases in vascular stiffness and wave reflections (Heffernan et al. 2013). Previous studies have reported no change in antegrade shear during this experimental manipulation and this is likely due to the manner of calculating antegrade shear as previous studies present both components of antegrade shear as a single composite measure (Schreuder et al. 2014, 2015; Zepetnek et al. 2014). With an increase in systolic antegrade shear and a reduction in diastolic antegrade shear, the net effect would be no change in total antegrade shear. There was no association between antegrade shear and microvascular oxygenation during the EXP compression condition. During conditions that alter perfusion pressure, microvascular perfusion can be uncoupled from convective oxygen delivery (Tucker et al. 2019). Thus, increases in SMO_2 in this setting may be the result of increased extraction to maintain oxygen consumption and not perfusion per se (Tucker et al. 2019).

Implications

Longer-term exposure to retrograde shear (2 weeks) via compression garments has been shown to reduce peripheral vascular endothelial function in young men (Thijssen et al. 2015). This is important to note as athletes commonly use compression garments to increase athletic performance and enhance recovery from strenuous exercise. Moreover, clinical populations may rely on compression socks to manage lymphedema or chronic venous insufficiency and these garments may apply compression pressures as high as 50–60 mmHg. Whether longer-term exposure to increased retrograde shear has a detrimental effect on microvascular oxygenation will require additional study.

Limitations

We defined ET a priori as men participating in Division I collegiate running. We did not assess time spent in moderate-to-vigorous physical activity with accelerometry or objective fitness utilizing metabolic gas exchange to measure aerobic capacity. Rather, we utilized a self-report physical activity questionnaire to determine physical activity levels which has inherent limitations (Helmerhorst et al. 2012). Although the validity of participants' subjective physical activity levels may be called into question, the objective physiological data supports the ET status of our participants

(e.g. larger SFA diameters, lower resting HR, higher SV, lower aortic PWV and AIx etc.).

This was a cross-sectional study that only examined young, healthy men, which restricts generalizations to other populations. Previous work done by Augustine et al. examined young women and found that there were no differences in the retrograde shear profile of exercise-trained and untrained females (Augustine et al. 2016). Yet, participants in this study were not exposed to external compression. Tremblay et al. recently noted sex differences in the acute impact of retrograde shear on vascular endothelial function (Tremblay et al. 2018). More research is needed to determine if use of external compression in females might reveal differences in retrograde shear not apparent during rest.

We chose to perform Doppler-ultrasound measures once during compression to confirm the stimulus. We visually confirmed the change in the shear profile throughout the 30-min protocol and our manipulation was successful at increasing retrograde shear. However, it is possible that we missed important and insightful information regarding the kinetics of change in shear patterns during the initial moments of external compression. High aerobic fitness and/or habitual exercise training may not only enhance the rapid vasodilatory response to muscle contraction (Hughes et al. 2016), it may also enhance the vasoconstrictor response to low-flow induced by external compression (Bell et al. 2017) thus increasing the overall vasoactive range. The habitual lower shear in the athlete's artery (Dinenno et al. 2001) may sensitize the endothelium to epochs of low shear. Thus, the athlete's artery may habituate to prolonged compression, altering the shear profile over time. Future studies should be carried out exploring the time course of potential changes in shear patterns across the 30 min of external compression.

The sample size was relatively small. While we were adequately powered to detect a condition effect for change in retrograde shear with 60 mmHg of compression (observed power 0.89 with a partial $\eta^2 = 0.34$), our study may have been underpowered to detect a group-by-condition interaction (observed power 0.32 with a partial $\eta^2 = 0.12$). Finally, SFA IMT was measured using manual calipers, which is sensitive to measurement error. Use of automated wall-tracking measures may improve accuracy of measures, although reliability of manual versus automated methods are comparable (Freire et al. 2009) and automated approaches are not without their own inherent limitations (Nichols et al. 2016).

Conclusions

Taken together, our findings suggest that beneficial vascular remodeling (larger diameter and lower IMT) in ET men is associated with a more favorable peripheral shear profile (i.e. lower retrograde shear) during acute increases in retrograde shear induced by external compression. Our findings also suggest that acute manipulation of retrograde shear with external compression does not detrimentally impact microvascular oxygenation.

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