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ORIGINAL ARTICLE

Peripheral conduit and resistance artery function are improved following a single, 1-h bout of peristaltic pulse external pneumatic compression

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Abstract
Introduction External pneumatic compression (EPC) is being employed for a widening range of clinical and non-clinical populations. However, EPC devices vary markedly in treatment pressures, duty cycles and application sites, and the acute effects of whole limb, lower pressure EPC on peripheral vascular function have not been determined.
Purpose The purpose of this study was to determine the acute effects of a single bout of peristaltic pulse EPC on peripheral conduit and resistance artery function.
Methods Twenty ($n = 20$; males = 12 and females = 8) young and apparently healthy subjects (aged 26.1 ± 8.2 years) participated in this study. A sequential EPC device with five inflation zones arranged linearly and inflating distal to proximal along the lower limbs was employed with target inflation pressures of 70 mmHg for 1 h. Flow-mediated dilation (FMD) of the brachial and popliteal arteries was evaluated with ultrasound before and after EPC. Venous occlusion plethysmography was employed to evaluate limb blood flow at rest and during reactive hyperemia (RH) in the forearm (FBF) and calf (CBF) before and after EPC.
Results Peak RH CBF was increased by 9 % after EPC ($P < 0.05$), whereas peak RH FBF (−10 %) did not change significantly ($P > 0.25$). Normalized popliteal artery FMD post-EPC (2.24 ± 1.41) was significantly higher than pre-EPC (1.36 ± 0.67, $P = 0.015$) and post-sham (1.58 ± 0.86, $P = 0.032$) values. Similarly, normalized brachial artery FMD post-EPC (1.47 ± 0.32) was significantly higher than pre-EPC (1.11 ± 0.41, $P = 0.004$) and post-sham (0.99 ± 0.27, $P = 0.026$) values.
Conclusion Acutely, whole limb, lower pressure EPC improves conduit artery endothelial function systemically, but only improves RH blood flow locally (i.e., compressed limbs).

Keywords Endothelial function · External pneumatic compression · Flow-mediated dilation · Peripheral vascular function · Venous occlusion plethysmography

Abbreviations

6-Keto-PGF1α	6-Keto prostaglandin F1α
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
BP	Blood pressure
CAD	Coronary artery disease
CBF	Calf blood flow
CVC	Calf vascular conductance
eNOS	Endothelial nitric oxide synthase
EPC	External pneumatic compression
EECP	Enhanced external counterpulsation
FBF	Forearm blood flow
FMD	Flow-mediated dilation
aFMD	Absolute FMD

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%FMD	Relative FMD
nFMD	FMD normalized to shear rate
FVC	Forearm vascular conductance
IL-6	Interleukin-6
IPC	Intermittent pneumatic compression
mRNA	Messenger RNA
NO	Nitric oxide
PGI2	Prostacyclin
RH	Reactive hyperemia
SNS	Sympathetic nervous system
VOP	Venous occlusion plethysmography

Introduction

The clinical employment of high pressure external pneumatic compression (EPC) imparts remarkable benefits to both central and peripheral vascular function and has been investigated extensively (Braith et al. 2012). 'Enhanced' external counterpulsation (EECP) is a non-invasive, high pressure therapy that consists of three pneumatic compression cuffs applied to the lower limbs. During EECP, these cuffs are sequentially inflated, from distal to proximal, with target inflation pressures of 300 mmHg during cardiac diastole until rapid deflation immediately prior to systole. A single 45 min EECP session has been shown to acutely increase flow-mediated dilation (FMD) (Gurovich and Braith 2013), a surrogate of conduit artery endothelial function and nitric oxide bioavailability (Akhtar et al. 2006). Chronically, administration of EECP has been shown to elicit significant improvements in FMD of the brachial (Hashemi et al. 2008; Braith et al. 2010; Beck et al. 2014), femoral (Braith et al. 2010; Beck et al. 2014), and popliteal arteries (Martin et al. 2012). Moreover, resistance artery blood flow and reactivity (e.g., peak reactive hyperemic blood flow) is significantly improved following chronic EECP administration in clinical (coronary artery disease) and pre-clinical (abnormal glucose tolerance) patients (Braith et al. 2010; Avery et al. 2014; Martin et al. 2014).

EECP is expensive, not readily accessible, and requires medical supervision. Therefore, despite the benefits of EECP on vascular biology, novel EPC alternatives for augmenting peripheral vascular function are desirable, particularly for application to a wider range of health and disease states. Novel EPC devices diverge from the clinical platform in two main areas; (1) inflation pressures and (2) inflation/deflation cycling speeds. Alternative dynamic EPC typically produces appreciably lower inflation pressures (50–100 vs. 300 mmHg for EECP) and longer inflation/deflation timed cycles which occur over minutes and are not coordinated with the cardiac cycle.

Examples of lower pressure EPC therapy include intermittent pneumatic compression (IPC) in which compressive boots (covering the feet and ankles) are inflated with particular duty cycles. Although IPC application methods vary amongst studies in the literature, IPC has demonstrated some promise with regard to acute effects on vascular function. Indeed, in a rodent model, endothelial nitric oxide synthase (eNOS) mRNA expression and protein concentration have been shown to be significantly upregulated 4-h following a 1-h bout of IPC (Chen et al. 2002; Tan et al. 2006). However, in human application, although local hemodynamic shear stress is significantly increased during treatment, FMD and peak reactive hyperemic blood flow in the resistance arteries do not appear to be immediately improved following acute and chronic IPC application, respectively (Roseguini et al. 2010; Sheldon et al. 2012). In contrast, massage therapy, which is reasonably similar to EPC, has been shown to facilitate systemic improvements as brachial FMD is increased for up to 48 h following massage of the lower limbs (Franklin et al. 2014). Furthermore, massage therapy was shown to restore the deleterious effects of a bout of exercise-induced muscle injury on peripheral vascular function. Collectively, the changes following various forms of external compression are intriguing and warrant further investigation with other modes of compression/EPC.

Accordingly, the purpose of our study was to determine, for the first time, the acute effects of a single 1 h bout of peristaltic pulse EPC treatment on peripheral conduit and resistance artery vascular function. The EPC device employed herein was chosen specifically as it uses a novel peristaltic pulse compression waveform for sequential circumferential compression of the entire lower limb without the aid of a therapist. We hypothesized that application of EPC for 1 h at 70 mmHg would acutely improve surrogates of vascular function in the conduit and resistance vessels in both compressed (lower limbs) and non-compressed limbs (arms).

Methods

Subjects

Twenty ($n = 20$; males = 12 and females = 8) apparently healthy subjects were recruited from the local community through advertisement to participate in this crossover design study. All subjects reported for two separate visits, separated by at least 72 h, and were instructed to abstain from exercise and alcohol for 24 h and from caffeine for 12 h. All subjects reported to the laboratory at least 4 h post-prandial. In order to control for any potential diurnal

variation, all subjects reported for the study at the same time of day.

Study design

Two arms of the study were conducted independently with 10 subjects participating in each; (1) assessment of limb blood flow and peripheral resistance artery reactivity in the calf and forearm via venous occlusion plethysmography (VOP), and (2) assessment of FMD in peripheral conduit arteries (brachial and popliteal) using ultrasonography. VOP was employed as it provides a surrogate of endothelial function in the small, resistance arteries, and whole limb blood flow. Ultrasonography/FMD protocols were employed as it is considered a bioassay of nitric oxide bioavailability and can provide a surrogate of endothelial function and measures of blood velocity/shear rate in the muscular conduit arteries. Treatment (EPC or sham first) and limb measurement order (upper or lower limb assessment first before and after EPC and sham) was randomized for all subjects according to odd/even number assignments using the first and last integers of the respective subject's randomly generated 4-digit subject identification code. The study was approved by the Quinnipiac University Institutional Review Board, and written informed consent was obtained from all participants.

Procedures

Treatments: external pneumatic compression (EPC) and sham

A peristaltic pulse dynamic EPC device (NormaTec, Newton Center, MA, USA) was used for EPC treatments. The EPC device consists of two separate "leg sleeves" which contain five circumferential inflatable chambers (arranged linearly along the limb) encompassing the leg from the feet to the hip/groin. The "leg sleeves" are connected to an automated pneumatic pump at which target inflation pressures for each zone and the duty cycle can be controlled. However, the unit is commercially marketed with preprogrammed defaults for the duty cycle and recommended inflation pressure settings for recovery that we have previously investigated and described (Martin et al. 2015b). Therefore, in this study, we chose to use the recovery protocol recommended by the manufacturer with target inflation pressures of ~70 mmHg for each chamber. In brief, beginning with the most distal chamber, inflation occurs and the chamber "pulses" (slight variations in pressure) for ~1 min after which pressure is held constant (~70 mmHg) to prevent backflow. The same process then occurs in each of the next highest zones individually. Notably, a maximum of only two distal chambers is held at constant pressure to facilitate greater rest time (no compression) in each chamber. After the most proximal zone completes its cycle, all zones are completely deflated for approximately 30 s. This entire cycle of compression is repeated continuously over the course of a single 60 min treatment session.

The sham treatment condition consisted of application of the EPC "leg sleeves" and connection to the pneumatic pump, but was devoid of actual compression for 60 min. This protocol was employed to control for any thermogenic effect of wearing the leg sleeves as heat loss from the legs is likely affected. A lower pressure sham treatment was not employed given the potential for even very low pressures having an effect on the study outcomes.

Arm 1: forearm and calf blood flow and resistance artery reactivity

Following 15 min of rest in a supine position, brachial artery blood pressure (BP) and heart rate (HR) measurements were performed in triplicate using an automated oscillometric device (OMRON BP785, Omron Corporation, Kyoto, Japan), and an average of the measurements was used for resting values. Moreover, BP in the contralateral limb was assessed to assure that no significant between arm difference (defined as Δ 10 mmHg for systolic or diastolic BP) was present as this may suggest bilateral variability and only unilateral measures were employed herein (Martin et al. 2015a). Mean arterial pressure (MAP) was determined as diastolic BP + (pulse pressure/3).

Following BP measurement, forearm (FBF) and calf blood flow (CBF) at rest and during reactive hyperemia (RH) were determined independently by VOP (EC-6, D.E. Hokanson, Inc., Bellevue, WA, USA) using calibrated indium-gallium strain gauges, as previously described (Wilkinson and Webb 2001). Briefly, strain gauges were applied to the widest part of the non-dominant forearm or calf after limb circumference measurement with arms or legs elevated slightly above heart level. To measure FBF and CBF, an upper arm or thigh cuff was inflated to 50 mmHg for 7 s each 15 s measurement cycle using a rapid cuff inflator (EC 20; DE Hokanson Inc.) to prevent venous outflow (Patterson and Whelan 1955; Hokanson et al. 1975). One minute before each measurement, a wrist or ankle cuff was inflated to 200 mmHg of constant pressure to occlude hand or ankle circulation during FBF or CBF measurements. Twelve plethysmograph measurements (over a total of 3 min) were averaged for baseline BF values.

Endothelium-dependent FBF and CBF were measured following 5 min of upper arm or thigh arterial occlusion during RH of the forearm or calf. A cuff was placed on the upper arm or thigh 5 cm above the antecubital or 10 cm above the popliteal fossa, respectively. After baseline FBF

and CBF measurements, the cuff was rapidly inflated to 200 mmHg for 5 min and then rapidly deflated. Peak FBF and CBF were recorded as the highest BF observed during 3 min of RH, and total FBF and CBF were recorded as the area under the time curve after baseline BF was subtracted (Meredith et al. 1996).

The plethysmography output signal was transmitted to the Non-Invasive Vascular Program (NIVP3) calibrated software program (DE Hokanson Inc.) and BF was expressed as milliliters (mL) per minute per deciliter (dL) of forearm or calf tissue (mL/min/dL tissue).

Arm 2: peripheral conduit artery flow-mediated dilation (FMD)

The FMD technique was used to determine endothelial-dependent reactivity in the brachial and popliteal arteries. After lying quietly for 15 min (baseline) and immediately after 1-h of EPC or sham, a 5–12 MHz multi-frequency linear phase array ultrasound transducer (SonoScape S2, SonoScape Medical Corp, Shenzhen, China) was used to image the right brachial artery longitudinally. Resting baseline brachial artery diameters and blood velocity were captured with the transducer placed 3–5 cm above the antecubital fossa for 1 min each (using B and Doppler mode, respectively). After obtaining baseline measures, RH was produced by inflating a BP cuff placed on the upper forearm, 1–2 cm below the elbow, for 5 min at 200 mmHg. The transducer was manually held in the same position for the duration of cuff inflation, and brachial artery diameter was measured continuously. At ~10 s prior to and for 20 s following cuff release, blood velocity was measured in Doppler mode. Thereafter, imaging switched back to B-mode for continuous measurement of brachial artery diameter for an additional 160 s. Due to the appreciable difference in image quality during duplex imaging using the SonoScape S2, simultaneous diameter and velocity measurements were not performed.

Popliteal artery FMD was measured similarly, except the subjects were lying prone and had a small foam pad placed under the ankle. Popliteal artery diameters and blood velocity were obtained with the transducer placed 2–3 cm above the bifurcation and the occlusion cuff was placed around the leg, 5–8 cm distal to the popliteal fossa, for 5 min at 200 mmHg.

Ultrasound video was digitally recorded (at 5 frames/s) directly to a digital storage device via video interface (Pinnacle, Avid Technology) for off-line electronic image analysis using automated FMD software (Brachial Analyzer for Research; Medical Imaging Applications LLC, Iowa). Vessel diameters were determined frame-by-frame via automatic edge detection software (Brachial Analyzer for Research) measuring the distance between the near and far wall of the intima. Blood velocity was determined via selection of a region of interest around the Doppler waveform, and a trace of the velocity–time integral was used to calculate mean velocity for each cardiac cycle. Vessel diameters and blood flow velocities were determined using a 3-s moving average. Previous investigations have employed this approach and demonstrated no difference between continuous measurement of vessel diameter at 5 frames/s compared to R-wave gated frames (Padilla et al. 2009). Brachial FMD was calculated as absolute (aFMD, reported in mm) and relative (%FMD, reported in %) peak change in brachial artery diameter in response to the hyperemic stimulus. Because dilation also depends on the resultant hyperemic flow stimulus, all measurements of peak %FMD were also normalized (nFMD) to the average mean shear rate observed during the first 20 s following cuff release ($100 \times$ %FMD $\times$ average mean shear rate^{-1}). In the absence of blood viscosity, shear rate is measured by the following equation: shear rate (s^{-1}) $= 4 \times$ mean blood velocity (cm/s) $\times$ diameter (cm^{-1}).

Statistical analysis

All data were tested for normal distribution using the Shapiro–Wilk test for normality. An alpha level of $P \leq 0.05$ was required for statistical significance. A repeated measures 2-way ANOVA was used to evaluate the continuous primary dependent variables associated with both arms of the study. When a significant treatment-by-time interaction was observed, within-treatment (e.g., pre-EPC vs. post-EPC) and between-treatment (e.g., post-EPC vs. post-sham) comparisons were performed using Student's paired *t* tests to analyze differences between time points. All statistical analyses were performed using IBM SPSS Statistics 22 for Windows (Chicago, IL). All data are reported as mean ± SD.

Results

Arm 1: acute peristaltic EPC improves local resistance vessel reactivity

Subject characteristics for study arm 1 are presented in Table 1. In study arm 1, no main effects (i.e., time or treatment) or time $\times$ treatment interaction were observed for HR and MAP. HR and MAP values from pre- to post-1-h of EPC were 61 ± 8 to 58 ± 8 bpm and 84 ± 8 to 86 ± 8 mmHg, respectively. Values from pre- to post-1-h of sham were 61 ± 8 to 58 ± 7 bpm and 83 ± 10 to 84 ± 8 mmHg for HR and MAP, respectively.

Resting, peak RH and total RH CBF values pre- and post-EPC and sham treatment are presented in Fig. 1

Table 1 Subject characteristics for study arm 1 (venous occlusion plethysmography)

Variables	M ± SD	Range
Arm 1: venous occlusion plethysmography		
Number of males	6	–
Number of females	4	–
Age (years)	27.0 ± 10.1	18.7–50.34
Height (cm)	169 ± 8	159–182
Weight (kg)	73.1 ± 14.0	53.2–99.5
BMI (kg/m^2)	25.5 ± 4.2	20.8–34.9
Arm 2: flow-mediated dilation		
Number of males	6	–
Number of females	4	–
Age (years)	25.3 ± 6.8	19.7–38.11
Height (cm)	173 ± 14	155–198
Weight (kg)	74.2 ± 18.7	49.5–98.6
BMI (kg/m^2)	24.3 ± 3.1	20.1–29.6

(panels a–c). For resting CBF (Fig. 1a), a significant main effect of time ($P = 0.013$) was observed, but no main effect of treatment or time × treatment interaction. No main effects were observed for peak RH CBF, though there was a significant treatment × time interaction ($P = 0.017$; Fig. 1b). Post hoc analysis revealed that EPC significantly increased peak RH CBF compared to baseline (+2.4 ± 0.9 mL/min/dL tissue; $P = 0.023$), but sham had no significant effect (−0.8 ± 0.8 mL/min/dL tissue; $P = 0.361$). The higher peak RH CBF observed post-EPC compared to post-sham did not reach statistical significance ($P = 0.076$). No significant main effects or interaction were observed for total RH CBF (Fig. 1c).

Resting, peak RH and total RH FBF values pre- and post-EPC and sham treatment are presented in Fig. 1 (panels d–f). A significant main effect of treatment ($P = 0.011$) and time ($P = 0.010$) was observed for resting FBF (Fig. 1d), but no interaction was present. No significant

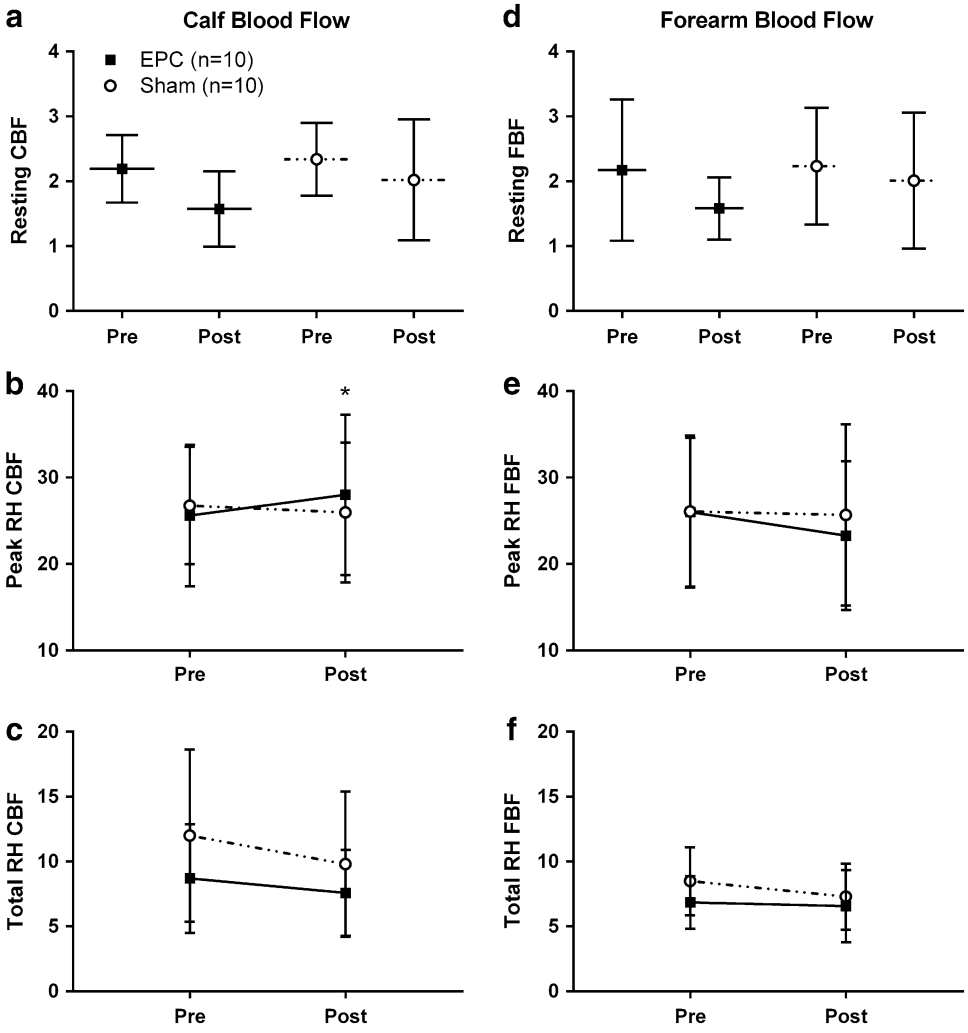

Fig. 1 Venous occlusion plethysmography parameters of limb blood flow in the calf (Panels **a**–**c**) and forearm (Panels **d**–**f**) pre- and post-1 h of external pneumatic compression (EPC, *black square*) and sham (*white circle*) treatment. Data are presented as mean absolute values in mL/min/dL of tissue ± SD. **a** Resting calf blood flow (CBF), **b** peak reactive hyperemia (RH) CBF, **c** total RH CBF, **d** resting forearm blood flow (FBF), **e** peak RH FBF, and **f** total RH FBF. When a significant time × treatment interaction was observed, post hoc tests were performed using Student's paired *t* tests. *Significantly different pre-EPC versus post-EPC ($P < 0.05$)

Table 2 Brachial and popliteal artery flow-mediated dilation characteristics at baseline and after EPC or sham

	EPC ($n = 10$)		Sham ($n = 10$)	
	Pre	Post	Pre	Post
HR (bpm)	60 ± 10	59 ± 12	60 ± 10	58 ± 8
MAP (mmHg)	87 ± 12	87 ± 12	86 ± 10	86 ± 8
Popliteal artery				
Mean blood velocity (cm/s)				
Resting	8.0 ± 1.2	8.0 ± 2.0	7.9 ± 3.0	8.2 ± 2.7
Hyperemia	28.2 ± 6.9	24.4 ± 6.5*	26.5 ± 4.9	28.6 ± 9.2
Mean shear rate (s^{-1})				
Resting	62.5 ± 7.1	64.6 ± 24.3	63.0 ± 27.5	65.8 ± 28.5
Hyperemia	222 ± 64	199 ± 74	207 ± 44	222 ± 67
Absolute FMD (mm)	0.14 ± 0.05	0.19 ± 0.06**,††	0.16 ± 0.07	0.16 ± 0.05
Brachial artery				
Mean blood velocity (cm/s)				
Resting	15.1 ± 5.4	12.5 ± 5.3	15.2 ± 5.7	11.3 ± 3.7
Hyperemia	50.9 ± 8.2	45.2 ± 16.5	55.7 ± 9.3	49.7 ± 17.6
Mean shear rate (s^{-1})				
Resting	174 ± 70	136 ± 50	169 ± 66	120 ± 32
Hyperemia	583 ± 185	493 ± 179	624 ± 146	528 ± 137
Absolute FMD (mm)	0.22 ± 0.09	0.26 ± 0.08†	0.21 ± 0.07	0.19 ± 0.06

Data are expressed as mean ± SD. Mean blood velocity and shear rate were measured at baseline and during the first 20 s following cuff release; FMD is flow-mediated dilation expressed a peak absolute dilation (in mm). When a significant time × treatment interaction was observed, post hoc analysis was conducted using Student's paired *t* tests

*,** Significantly different pre-EPC versus post-EPC ($P < 0.05$ and $P < 0.01$, respectively)

†,†† Significantly different post-EPC versus post-sham ($P < 0.05$ and 0.01, respectively)

main effects or interaction were observed for peak and total RH FBF (Fig. 1e, f).

Arm 2: acute peristaltic EPC elicits systemic improvements in conduit artery flow-mediated dilation

Subject characteristics for arm 2 of the study are presented in Table 1. Subjects' vitals pre- and post-EPC and sham treatment are presented in Table 2. Similar to study arm 1, in study arm 2 HR and MAP did not change significantly following a 1-h EPC or sham treatment. Moreover, no significant differences between conditions (EPC vs. sham) in pre- or post-treatment measures of HR and MAP were observed.

There were no significant main effects or interaction for resting popliteal artery diameter (Fig. 2a). Similarly, no significant main effects, nor interaction, were observed for resting mean blood velocity and shear rate in the popliteal artery (Table 2). Although no significant main effects were observed for mean RH blood velocity and shear rate, a significant interaction was present ($P = 0.028$ and 0.042, respectively; Table 2). Post hoc analysis revealed a significant 3.7 ± 4.8 cm/s decrease in mean RH blood velocity following EPC ($P = 0.037$), but the 23.7 ± 40.8 s^{-1} decrease in mean RH shear rate following EPC was not statistically significant ($P = 0.099$). The moderate increases in mean RH blood velocity (+2.1 ± 6.7 cm/s) and shear rate (+15.1 ± 52.0 s^{-1}) following sham treatment were not statistically significant ($P = 0.349$ and 0.384, respectively). Analysis of aFMD (Table 2), %FMD (Fig. 2b) and nFMD (Fig. 2c) responses in the popliteal artery demonstrated a significant main effect of time ($P = 0.001$, 0.001, and 0.042, respectively) and significant time × treatment interactions ($P = 0.009$, 0.005, and 0.010, respectively). However, no significant main effect of treatment was observed for aFMD, %FMD, and nFMD in the popliteal artery. Post hoc analysis revealed that EPC treatment significantly increased popliteal artery aFMD (+5.3 ± 3.6 mm; $P = 0.001$), %FMD (+1.03 ± 0.53 %; $P < 0.001$), and nFMD (+0.88 ± 0.93; $P = 0.015$), but there was no significant effect of sham treatment ($P = 0.658$, 0.810 and 0.788 for aFMD, %FMD and nFMD, respectively). In addition, post-treatment values (i.e., post-EPC vs. post-sham) for popliteal artery aFMD, %FMD, and nFMD were all found to be significantly higher following the EPC treatment condition ($P = 0.004$, 0.001 and 0.032, respectively; Table 2; Fig. 2b, c).

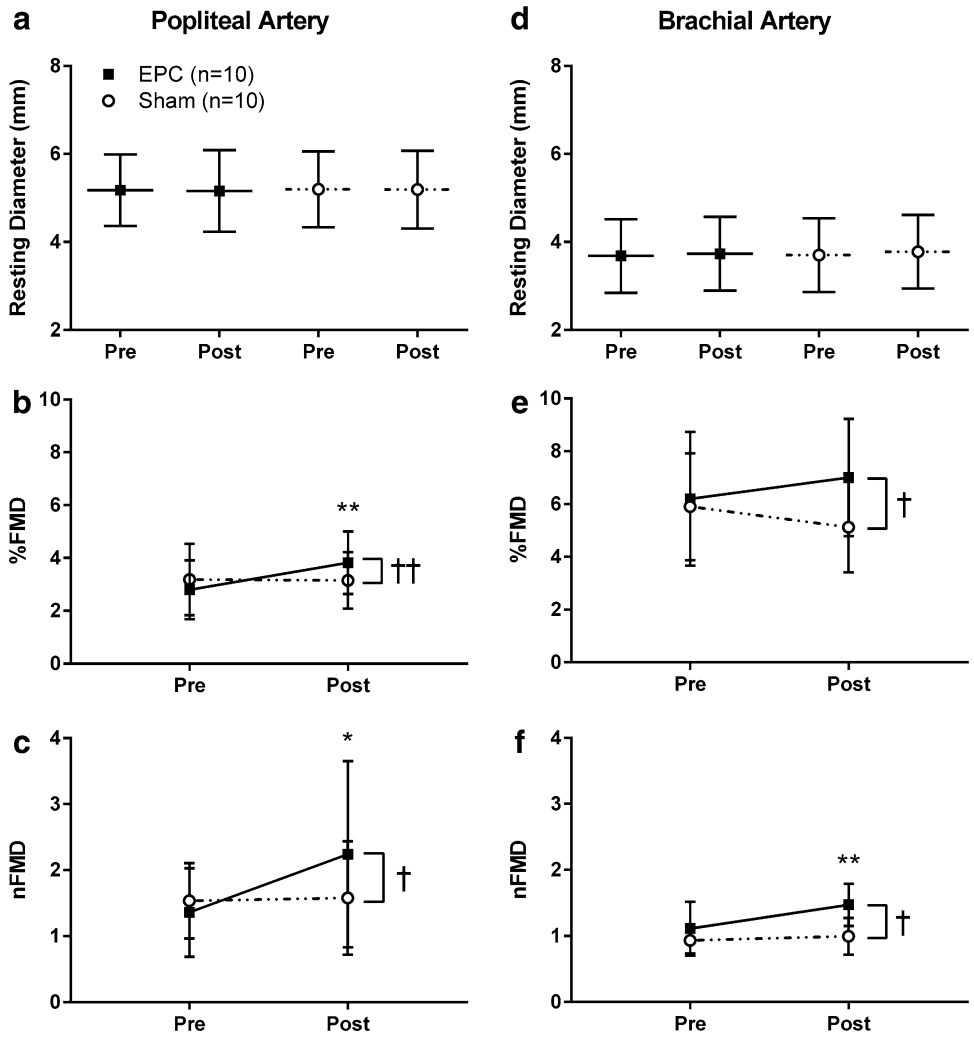

Fig. 2 Parameters derived from flow-mediated dilation (FMD) measurement in the popliteal (Panels **a**–**c**) and brachial (Panels **d**–**f**) arteries pre- and post-1 h of external pneumatic compression (EPC, *black square*) and sham (*white circle*) treatment. **a** Resting popliteal artery diameter in mm, **b** popliteal relative peak FMD (%FMD, expressed as a *percentage*), **c** popliteal normalized peak FMD (nFMD), **d** resting brachial artery diameter in mm, **e** brachial %FMD, and **f** brachial nFMD. Data are presented as mean values ± SD. When a significant time × treatment interaction was observed, post hoc tests were performed using Student's paired *t* tests. *, **Significantly different pre-EPC versus post-EPC ($P < 0.05$ and 0.01, respectively); †, ††Significantly different post-EPC versus post-sham ($P < 0.05$ and 0.01, respectively)

Resting brachial artery diameter values pre- and post-EPC and sham treatment are presented in Fig. 2d. No significant main effects, nor interaction, were observed for resting brachial artery diameter. Similarly, no significant main effects, nor interaction, were observed in the brachial artery for resting mean blood velocity and mean RH blood velocity and shear rate. However, a significant main effect of time was observed for brachial artery resting shear rate ($P = 0.029$; Table 2) though there was no main effect of treatment or time × treatment interaction. A significant time*treatment interaction, but no main effects, was observed for brachial artery aFMD and %FMD ($P = 0.010$ and 0.007, respectively; Table 2; Fig. 2e). In addition, significant main effects of time ($P = 0.017$) and treatment ($P = 0.035$), and their interaction ($P = 0.009$), were observed for brachial artery nFMD (Fig. 2f). Post hoc analysis revealed no significant effects of sham treatment on brachial artery aFMD (−0.24 ± 0.46 mm, $P = 0.142$), %FMD (−0.78 ± 1.16 %, $P = 0.062$) or nFMD (+0.05 ± 0.31, $P = 0.587$). EPC treatment significantly increased brachial artery nFMD (+0.36 ± 0.30; $P = 0.004$), but increases in brachial artery aFMD (+0.33 ± 0.54 mm; Table 2) and %FMD (+0.80 ± 1.22 %; Fig. 2e) following EPC did not reach statistical significance ($P = 0.088$ and 0.067, respectively). However, post-treatment values (i.e., post-EPC vs. post-sham) for brachial artery aFMD (Table 2), %FMD (Fig. 2e), and nFMD (Fig. 2f) brachial artery FMD were all found to be significantly higher following the EPC treatment condition ($P = 0.019$, 0.030, and 0.026, respectively.

Discussion

The potential of lower pressure EPC devices in historically amenable areas (e.g., vascular dysfunction) and/or in new paradigms is intriguing. To date, however, studies have not fully elucidated the mechanisms of action or the acute effects of lower pressure EPC treatment on peripheral

conduit and resistance artery vascular function. The principle findings of the present study demonstrate, for the first time, that a single 1 h bout of peristaltic pulse EPC with low-target inflation pressures acutely improves local resistance vessel reactivity (i.e., peak blood flow) and systemically improves FMD, a bioassay of nitric oxide bioavailability, in the peripheral conduit arteries.

The effects of high pressure EPC have been well characterized (Braith et al. 2012). One of the primary mechanisms of action proposed for high pressure EPC-mediated improvements in vascular biology is the acute increase in hemodynamic shear stress. During high pressure EPC that is synchronized to the cardiac cycle (i.e., EECP; inflation during diastole, deflation immediately prior to systole), hemodynamic shear stress has been shown to increase by 402 % in the femoral artery of the lower limb and by 75 % in the brachial artery of the upper limb (Gurovich and Braith 2013). With the EPC device used herein, target inflation pressures are substantially lower, asynchronous to the cardiac cycle, and do not alter blood flow in the upper extremities (Martin and Laughlin 2013). Given the encapsulating nature of the EPC "leg sleeves" direct measurement of popliteal and/or femoral blood flow during treatment was not feasible in the present study. However, it is reasonable to assume that during compression, blood flow dynamics are substantially altered and that hemodynamic shear stress increases in the lower limbs during the cycle of all zone deflation (e.g., mild-moderate reactive hyperemic response) which follows the preceding 5 min of sequential compression.

Regardless, although changes in lower limb hemodynamics could explain acute increases in popliteal artery FMD, the upstream increase in the non-compressed brachial artery nFMD and significantly higher post-EPC %FMD response compared to post-sham was somewhat surprising (Fig. 2). Massage therapy of the lower limbs has demonstrated similar benefits systemically (Franklin et al. 2014) and one possible explanation could include compression-mediated release of myokines. Specifically, interleukin-6 (IL-6), a myokine known to be released during skeletal muscle contraction (Pedersen and Febbraio 2008), may potentiate local vasodilation and/or systemic nitric oxide bioavailability as an AMP-activated protein kinase (AMPK) agonist in skeletal muscle and endothelial and smooth muscle cells (Horman et al. 2008; Fisslthaler and Fleming 2009). Interestingly, we also noted a substantial, though non-significant, decrease in brachial artery %FMD following sham treatment which contributes to the marked difference in post-treatment values between conditions (post-EPC vs. post-sham). We posit that our sham condition or exposure to repeated bouts of reactive hyperemia in a short period of time may be a contributing factor to this observation. Given the inherit differences in mechanical stress and tissue disruption between the two conditions (EPC vs. sham), it is possible that the response to ambient air exposure, and subsequent sympathetic nervous system (SNS) activity, could differ between the treatment conditions and affect the FMD response (Lind et al. 2002; Dyson et al. 2006). While we did measure changes in HR and MAP following treatments, this was performed immediately following conclusion of the treatment and these values may have changed with the delay to FMD data acquisition. Future investigations should continuously monitor BP and HR (and/or more direct measures of sympathetic nervous system activity) to explore the possible effects of SNS on the effects observed herein.

Despite systemic improvements in FMD, resistance artery reactivity was only improved in the compressed limb. In study arm 1, we observed a significant increase in peak RH CBF, but not peak RH FBF, following a 1 h bout of EPC. Interestingly, the observed increase in peak RH CBF was not paralleled by an increase in RH blood velocity in the popliteal artery following EPC in study arm 2. In fact, we observed a decrease (13 %) in mean blood velocity in the 20 s post-occlusion during the popliteal artery FMD protocol following a single bout of EPC (post-EPC vs. pre-EPC). Reasons for this discrepancy may include, but are not limited to, occlusion cuff positioning with the VOP and FMD techniques, changes in vessel diameters during the RH period (i.e., vasodilation) not detected by the FMD methods employed herein, and metabolic factors. Indeed, with VOP, the occlusion cuff is placed upstream of the measurement site, whereas in the FMD protocol it is placed downstream of the measurement site. This significantly alters the tissue deficit and stimulus for RH, as upper limb occlusion has been shown to elicit a greater RH response (Berry et al. 2000). Thus, it is possible that the greater RH stimulus allowed for the detection of a more subtle difference in post-occlusion RH during VOP. However, the directionality of the measures requires further investigation.

Peak RH limb blood flow measured via plethysmography is thought to be determined by both myogenic autoregulation (Shepherd 1983) and metabolic factors, with vasodilatory prostaglandins playing a primary role in the observed peak blood flow rate during hyperemia, but not NO (Engelke et al. 1996; Joyner et al. 2001). Clifford and colleagues have demonstrated that mechanical compression of isolated rat soleus feed arteries elicits dilation that has both endothelium-dependent and independent contributions which suggests a role for metabolic factors (e.g., the vasodilatory prostaglandin, prostacyclin (PGI_2) (Clifford et al. 2006). Moreover, cultured endothelial cells demonstrate increased PGI_2 synthetic capacity in response to cyclic strain (Sumpio and Banes 1988) and shear stress (Hanada et al. 1999; Osanai et al. 2000). This suggests that PGI_2 production and/or potential may be increased as a result

of EPC interventions. Indeed, circulating concentrations of 6-keto prostaglandin F1α (6-keto-PGF1α), a weaker, vasodilatory, active metabolite of PGI_2, have been shown to increase acutely in response to IPC (Guyton et al. 1988). In addition, chronic application of EECP has demonstrated marked increases in circulating concentrations of 6-keto-PGF1α as well as decreases in circulating cytokines (Braith et al. 2010; Martin and Braith 2012). Therefore, it is possible that local concentrations and synthetic capacity of vasoactive prostaglandins contribute to the improved peak RH CBF response seen in the current study. Moreover, since mechanical deformation of the vasculature and muscle tissue and alteration of hemodynamics only occurred in the lower limbs, it may explain the lack of significant findings in the forearm following lower limb EPC.

Massage is commonly thought to acutely increase limb blood flow to the treated area, but this remains controversial in the literature (Munk et al. 2012; Weerapong et al. 2012). In the present study, no difference in resting blood flow was observed in either limb (arm or leg) following EPC treatment. In fact, for resting CBF, there was a main effect of time suggesting lesser resting limb blood following EPC and sham, independent of treatment condition. Although a significant interaction was not observed ($P = 0.010$), EPC application appears to be a driving force behind this observation as resting CBF was decreased 28 % from baseline following EPC, whereas the decrease was less pronounced following sham compared to baseline (−10 %). We believe that there are couple plausible reasons for these findings. First, circumferential compression of the limbs for 1-h at 70 mmHg effectively "squeezes" fluid from the dermal layer ultimately contributing to a uniform decrease in skin thickness and total limb circumference that may be characterized by strain gauge plethysmography as a decrease in limb blood flow. Secondly, myogenic auto-regulation may occur following EPC treatment as a result of compression-mediated alteration of local hemodynamics during EPC resulting in an increase in local vascular resistance secondary to vasomotor tone during occluded venous outflow. Finally, several other plausible mechanisms are possible, but are outside the scope of this study and warrant further investigation.

FMD in the peripheral muscular arteries is tightly associated with future cardiovascular morbidity and mortality as evidenced by the work of Park and colleagues who demonstrated that FMD is an independent predictor of coronary heart disease (Park et al. 2011). Moreover, endothelium-dependent vasodilation (EDV), which is thought to be predictive of cardiovascular risk (Vita and Keaney 2002), during RH in the forearm has been shown to correlate highly with acetylcholine-induced EDV in both normotensive and essential hypertension persons (Higashi et al. 2001). Therefore, peak RH FBF and CBF are good non-invasive measures of EDV of resistance vasculature. Granted, the results of this study demonstrate simply the acute improvements in FMD systemically and peak RH CBF immediately following a single 1-h EPC treatment in an apparently healthy population. However, given the marked and long-lasting benefits of high pressure EPC (e.g., EECP) on vascular biology (Lawson et al. 1995; Braith et al. 2012), these findings are relevant and warrant further characterization of the duration and magnitude of the EPC-mediated improvements in FMD and to determine the potential for EPC application in the treatment of conditions associated with impaired vascular function. Moreover, in conjunction with the improved vasodilatory capacity observed in the compressed limbs, the ability of EPC to augment the vascular response to shear stress and/or metabolic demand potentiates greater nutrient delivery and metabolite clearance which is intriguing in the context of recovery and healing. Importantly, although vasomotor tone and/or peripheral resistance may have increased locally following EPC, maximal vasodilatory capacity was not impaired.

Experimental considerations and future directions

The findings of the present study may not be easily generalized to older adults or to patients with vascular disease. Moreover, two distinct populations were recruited for participation in each arm of the present study with a moderately large age range. However, sub-analysis of Pearson correlations between age and observed EPC-mediated changes in surrogates of endothelial function (e.g., post-EPC %FMD less pre-EPC %FMD) revealed no significant relationship ($P > 0.30$ for all). Therefore, our data would suggest that EPC-mediated improvements in FMD are not dependent upon age. The interpretation of our endothelium-dependent FMD data would be strengthened with accompanying vascular smooth muscle (i.e., endothelium independent) testing. Unfortunately, endothelium-independent vasodilation (via nitroglycerin) testing was not performed in the present study. However, it is unlikely that our observed acute improvements in FMD are due to heightened smooth muscle sensitivity to nitric oxide or altered cyclic guanosine monophosphate signaling during EPC treatment. Indeed, it was previously reported that effects of sublingual nitroglycerin spray, a nitric oxide donor acting directly on vascular smooth muscle cells, are unchanged in young and apparently healthy participants after massage therapy and endothelium-independent relaxation has been shown to be preserved in prehypertensives (Giannotti et al. 2010; Franklin et al. 2014). In addition, we did not measure shear rate area under the curve until peak FMD which may under represent nFMD measures (Pyke et al. 2004). However, the methods employed herein were performed, in part, for consistency with our previously published work

on the effects of EPC interventions on FMD (Braith et al. 2010; Martin et al. 2012; Beck et al. 2014). Finally, indices of sympathetic nervous system (SNS) activity were not measured in the present study, and consequently the effects of SNS modulation in response to EPC cannot be ruled out. Future studies with larger randomized samples, utilizing high versus low pressure therapy should be performed to elucidate the vascular impact of the non-clinical use of EPC.

Conclusions

A low pressure, peristaltic EPC device acutely improves conduit artery endothelial function systemically as evidenced by increases in brachial and popliteal FMD. Further, increases in peak CBF during RH suggest that these improvements may extend to the resistance arteries. The observations presented herein suggest that EPC may be a viable alternative for improving both peripheral conduit and resistance artery blood flow chronically after continued treatment.

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Conflict of interest The authors have no conflicts of interest to disclose.

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