Unilateral application of an external pneumatic compression therapy improves skin blood flow and vascular reactivity bilaterally

Jeffrey S Martin [Corresp., 1, 2] , Allison M Martin [1] , Petey W Mumford [2] , Lorena P Salom [2] , Angelique N Moore [1] , David D Pascoe [2]

[1] Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine - Auburn Campus, Auburn, United States

[2] School of Kinesiology, Auburn University, Auburn, United States

Corresponding Author: Jeffrey S Martin
Email address: jmartin@auburn.vcom.edu

Background: We sought to determine the effects of unilateral lower-limb external pneumatic compression (EPC) on bilateral lower-limb vascular reactivity and skin blood flow.

Methods: Thirty-two completed this two-aim study. In AIM1 (n=18, age: 25.5±4.7 years; BMI: 25.6±3.5 kg/m^2), bilateral femoral artery blood flow and reactivity (flow mediated dilation [FMD]) measurements were performed via ultrasonography at baseline (PRE) and immediately following 30-min of unilateral EPC treatment (POST). AIM2 (n=14, age: 25.9±4.5; BMI: 27.2±2.7 kg/m^2) involved 30-min unilateral EPC (n=7) or sham (n=7) treatment with thermographic bilateral lower-limb mean skin temperature (MST) measurements at baseline, at 15-min of treatment (T15) at 0, 30 and 60-min (R0, R30, R60) following treatment.

Results: Comparative data herein are presented as mean ± 95% confidence interval. AIM1: No significant effects on total reactive hyperemia blood flow were observed for the treated (i.e., compressed) or untreated (i.e., non-compressed) leg. A significant effect of time, but no time*leg interaction, was observed for relative FMD indicating higher reactivity bilaterally with unilateral EPC treatment (FMD: +0.41 ± 0.09% across both legs; p<0.05). AIM2: Unilateral EPC treatment was associated with significant increases in whole-leg MST from baseline during (T15: +0.63±0.56 °C in the visible untreated/contralateral leg, p<0.025) and immediately following treatment (i.e., R0) in both treated (+1.53 ± 0.59 °C) and untreated (+0.60 ± 0.45 ° C) legs (p<0.0125). Across both legs, MST remained elevated with EPC at 30-min post-treatment (+0.60 ± 0.45 °C; p<0.0167) but not at 60-min post (+0.27 ± 0.46 °C; p=0.165). Sham treatment was associated with a significant increase in the treated leg immediately post-treatment (+1.12 ± 0.31 °C; p<0.0167), but not in the untreated leg (-0.27 ± 0.12 °C). MST in neither the treated or untreated leg were increased relative to baseline at R30 or R60 (p>0.05). Finally, during treatment and at all post-treatment time points (i.e., R0, R30 and R60), independent of treatment group (EPC vs. sham), there was a significant effect of region. The maximum increase in MST was observed at the R0 time point and was significantly (p<0.05) larger in the thigh region (+1.02 ± 0.31 °C) than the lower-leg (+0.47 ± 0.29 °C) region. However, similar rates of MST decline from R0 in the thigh and lower leg regions were observed at the R30 and R60 time points.

Discussion: Unilateral EPC may be an effective intervention for increasing skin blood flow and/or peripheral conduit vascular reactivity in the contralateral limb. While EPC was effective in increasing whole-leg MST bilaterally, there appeared to be a more robust response in the thigh compared to the lower-leg. Thus, proximity along the leg may be an important consideration in prospective treatment strategies.

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5 Moore[1], David D. Pascoe[2]
6
7 [1]Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine-Auburn
8 Campus, Auburn, AL USA 36832

9 [2]School of Kinesiology, Auburn University, Auburn, AL USA 36849;
10
11 Corresponding Author:

12 Jeffrey S. Martin, Ph.D.
13 Department of Biomedical Sciences
14 Edward Via College of Osteopathic Medicine - Auburn Campus
15 910 S. Donahue Drive
16 Auburn, AL USA 36832
17 Email: jmartin@auburn.vcom.edu

18   **Abstract**

19   **Background**: We sought to determine the effects of unilateral lower-limb external pneumatic

20   compression (EPC) on bilateral lower-limb vascular reactivity and skin blood flow.

21   **Methods**: Thirty-two completed this two-aim study. In AIM1 (n=18, age: 25.5±4.7 years; BMI:

22   25.6±3.5 kg/m^2), bilateral femoral artery blood flow and reactivity (flow mediated dilation

23   [FMD]) measurements were performed via ultrasonography at baseline (PRE) and immediately

24   following 30-min of unilateral EPC treatment (POST). AIM2 (n=14, age: 25.9±4.5; BMI:

25   27.2±2.7 kg/m^2) involved 30-min unilateral EPC (n=7) or sham (n=7) treatment with

26   thermographic bilateral lower-limb mean skin temperature (MST) measurements at baseline, at

27   15-min of treatment (T15) at 0, 30 and 60-min (R0, R30, R60) following treatment.

28   **Results**: Comparative data herein are presented as mean ± 95% confidence interval. AIM1: No

29   significant effects on total reactive hyperemia blood flow were observed for the treated (i.e.,

30   compressed) or untreated (i.e., non-compressed) leg. A significant effect of time, but no

31   time*leg interaction, was observed for relative FMD indicating higher reactivity bilaterally with

32   unilateral EPC treatment (FMD: +0.41 ± 0.09% across both legs; $p<0.05$). AIM2: Unilateral

33   EPC treatment was associated with significant increases in whole-leg MST from baseline during

34   (T15: +0.63±0.56 °C in the visible untreated/contralateral leg, $p<0.025$) and immediately

35   following treatment (i.e., R0) in both treated (+1.53 ± 0.59 °C) and untreated (+0.60 ± 0.45 °C)

36   legs ($p<0.0125$). Across both legs, MST remained elevated with EPC at 30-min post-treatment

37   (+0.60 ± 0.45 °C; $p<0.0167$) but not at 60-min post (+0.27 ± 0.46 °C; $p=0.165$). Sham treatment

38   was associated with a significant increase in the treated leg immediately post-treatment (+1.12 ±

39   0.31 °C; $p<0.0167$), but not in the untreated leg (-0.27 ± 0.12 °C). MST in neither the treated or

40   untreated leg were increased relative to baseline at R30 or R60 ($p>0.05$). Finally, during

41   treatment and at all post-treatment time points (i.e., R0, R30 and R60), independent of treatment

42   group (EPC vs. sham), there was a significant effect of region. The maximum increase in MST

43   was observed at the R0 time point and was significantly (p<0.05) larger in the thigh region

44   (+1.02 ± 0.31 °C) than the lower-leg (+0.47 ± 0.29 °C) region. However, similar rates of MST

45   decline from R0 in the thigh and lower leg regions were observed at the R30 and R60 time

46   points.

47   **Discussion**: Unilateral EPC may be an effective intervention for increasing skin blood flow

48   and/or peripheral conduit vascular reactivity in the contralateral limb. While EPC was effective

49   in increasing whole-leg MST bilaterally, there appeared to be a more robust response in the thigh

50   compared to the lower-leg. Thus, proximity along the leg may be an important consideration in

51   prospective treatment strategies.

Introduction

Chronic wound care therapies often target improvements in local blood flow. However, one of the difficulties in the treatment of chronic wounds is the pain and complication associated with direct wound contact. Interventions aiming to circumvent this problem that, in part, aim to improve blood flow and healing in chronic wounds include vacuum-assisted closure, local tissue warming and non-contact radiant bandages. However, the potential for a whole-leg, dynamic, external pneumatic compression (EPC) therapy applied to a non-affected limb has not been explored. We have previously demonstrated that a single treatment with a low pressure (≤ 100 mmHg) EPC device (NormaTec Pro, NormaTec, Newton Center, MA, USA) acutely improves reactivity in the peripheral conduit and resistance vasculature of both compressed (i.e. legs) and non-compressed (i.e. arms) limbs (Martin et al. 2015a). Moreover, we have shown that local concentration of the stable metabolites of nitric oxide (NO) are increased in skeletal muscle biopsy samples following a 1-h bout of EPC (Kephart et al. 2015). Nevertheless, it has yet to be determined what effect unilateral EPC treatment would have on the contralateral limb. Recently, Credeur et al showed that unilateral treatment for 1-h with intermittent pneumatic compression (IPC) at target inflation pressures of 120 mmHg in spinal cord injury patients acutely increases posterior-tibial artery reactivity in the treated (compressed) leg, but not the untreated leg (2017). However, the aforementioned EPC device used in our prior investigations differs markedly from IPC as it is more dynamic (peristaltic compression pattern), utilizes a different duty cycle, contains additional inflation chambers and encompasses the entire leg. Given the need for non-contact and easily accessible interventions that can effectively improve blood flow and endothelial health to facilitate chronic wound healing, as a first step, we sought to determine the effect of dynamic, whole-leg EPC applied to a single leg on vascular reactivity and blood flow in

75   both legs in a healthy population. We hypothesized that unilateral EPC treatment would

76   effectively improve vascular reactivity, as determined by flow mediated dilation (FMD; AIM1),

77   and skin microcirculation, as determined by dynamic infrared thermography mean skin

78   temperature (MST; AIM2), in both the treated (i.e., compressed/covered) and untreated (i.e.,

79   non-compressed/non-covered) legs. In addition, we hypothesized that the improvements in skin

80   blood flow in the limbs would be global (i.e., not segmental) in nature.

Materials & Methods

Participants

83   Twenty (N=20) and fourteen (N=14) apparently healthy persons were recruited from the

84   local community to participate in AIM1 and AIM2 of this study, respectively. All procedures

85   herein were approved by Auburn University's Institutional Review Board (Project #15-017

86   MR1502) and conformed to the standards set by the latest revision of the Declaration of

87   Helsinki. Written informed consent was obtained from all participants prior to their voluntary

88   participation in the study.

Study Design

AIM1

91   For AIM1, twenty persons (10 females, 10 males) reported for a single visit which was

92   comprised of baseline bilateral blood pressure and popliteal artery FMD measurements, 30-min

93   of unilateral EPC application with target inflation pressures of 70 mmHg, and a second set of

94   bilateral FMD measurements 30-min after completion of the EPC treatment (POST). Bilateral

95   blood pressure measures were employed to identify any significant between arm variability

96   (defined as $\geq$ 10 mmHg for systolic or diastolic blood pressure) that may confound the results

97   (Martin et al. 2015b). In AIM1, two persons were identified as having significant between arm

98   blood pressure variability and were excluded from the study. Thus, the total number of

99   participants for AIM1 was eighteen (9 females, 9 males).

100   *AIM2*

101   For AIM2, fourteen participants (4 females, 10 males) reported for a single visit which

102   was comprised of baseline bilateral blood pressure and leg thermography measurements,

103   randomization to either 30-min of unilateral EPC treatment with target inflation pressures of 70

104   mmHg (n=7) or a 30-min sham treatment with unilateral positioning of an EPC "leg sleeve" with

105   no inflation/compression (n=7), and 60-min of post-EPC/sham thermography measurements.

106   Medical histories were assessed to confirm absence of medical conditions that might interfere

107   with the intervention(s) or outcomes (e.g. orthopedic injuries/surgeries, neurological issues,

108   diabetes, etc.). All participants were found to have regular thermal patterns in and between the

109   two legs per preliminary imaging and were free from significant between arm blood pressure

110   differences.

111   We have observed (informally) that the ultrasound/FMD procedure creates a slight,

112   transient (<5 minutes) increase in skin surface temperature specific to the area of contact. Thus,

113   separate trials were performed for each aim of the study.

114   **Procedures**

115   For both aims, all participants were instructed to abstain from exercise and alcohol for

116   24-h and from caffeine for 12-h. Additionally, all participants reported to the laboratory at least

117   2-h post-prandial and, in order to control for any potential diurnal variation, at the same time of

118   day.

119   *Brachial artery blood pressure*

120   For both aims, upon reporting to the laboratory, height and weight was measured using a

121   digital scale with height rod. Thereafter, following 15-min of supine rest, heart rate and brachial

122   artery systolic and diastolic blood pressure measurements were made at both arms using an

123   automated oscillometric device (OMRON BP785, Omron Corporation, Kyoto, Japan). The

124   average of the measurements at the left and right arm were recorded for characterization of blood

125   pressure.

126   *Flow Mediated Dilation*

127   In AIM1, following baseline heart rate and blood pressure measurement, popliteal artery

128   FMD was measured in both legs at baseline (i.e. PRE) and 30-min after (i.e., POST) unilateral

129   EPC application. The order of FMD measurement side (i.e. left or right leg) at baseline was

130   randomized for each subject, though that order was repeated for post-EPC measures.

131   FMD was used to determine endothelial-dependent reactivity in the popliteal arteries using

132   high resolution ultrasound (GE Logiq S7 R2 Expert) with a 3 to 12 MHz multi-frequency linear

133   phase array transducer. In addition, the reactive hyperemia response (i.e. peak and total reactive

134   hyperemia blood flow) during FMD procedures was determined for evaluation of microvascular

135   (i.e. arterioles and capillaries) reactivity. In brief, each artery was imaged longitudinally with the

136   transducer placed 3-8 cm below the popliteal fossa and held manually in the same position for

137   the duration of the measurements. Simultaneous measurement of artery diameter and blood

138   velocity was performed using duplex mode imaging (B-mode and Doppler) and video was

139   captured through a digital interface at 30 frames/second with real time analysis (FMD Studio

140   v2.8, QUIPU, Pisa, Italy). Resting measurements were captured for 1-min, reactive hyperemia

141   was produced by inflating a cuff placed on the calf for 5-min at 200 mmHg, and, after cuff

142   release, measurements were made for another 3-min. Vessel diameters were determined via

143   automatic edge detection software (FMD Studio) measuring the distance between the near and

144   far wall of the intima. Blood velocity was determined via selection of a region of interest around

145   the Doppler waveform and a trace of the velocity-time integral was used to calculate mean

146 velocity for each cardiac cycle. Shear rate was calculated as [(4*time averaged mean

147 velocity)/vessel diameter] and mean blood flow was calculated as [Π * (diameter/2)^2 * time

148 average mean velocity * 60].

149 FMD was calculated as absolute (aFMD; in mm) and relative (%FMD) peak change in

150 artery diameter in response to the hyperemic stimulus. Because dilation also depends on the

151 resultant reactive hyperemia shear rate stimulus, normalized FMD (nFMD) was calculated as the

152 ratio of %FMD to the shear rate area under the curve (AUC) to the time at which the maximal

153 diameter was observed (100*%FMD/shear rate AUC). Finally, peak reactive hyperemia blood

154 flow and total reactive hyperemia blood flow AUC were calculated from observed 5-s averages

155 during the reactive hyperemia period. AUC was determined using the sum of trapezoids method

156 after baseline values were subtracted.

157 *Thermography assessment of skin blood flow*

158 In AIM2, following 15-min of rest for equilibration to room/position conditions, infrared

159 images of the anterior aspect of the legs were taken using an infrared thermal imaging system

160 camera and processor (Computerized Thermal Imaging Inc., Ogden, UT, USA). Regions of

161 interest included the anterior thigh and lower leg. Leg region MST was determined from the

162 processor program that provides the average value of the pixels within the regions of interest.

163 Images were taken within the thermal lab where the temperature was maintained at 21±1.1 °C

164 (thermoneutral environment) and emissivity was set at 0.98. Images were taken at baseline, at

165 the mid-point of EPC/sham treatment (T15), immediately post-EPC/sham treatment (R0), and at

166 30- (R30) and 60-min (R60) following EPC/sham treatment. All participants wore gym shorts

167 and non-constrictive undergarments appropriate for complete visualization of the thighs. A

168 representative thermography image is presented in Fig. 1.

169 *External pneumatic compression (EPC) and sham*

170 For both AIM1 and AIM2, a dynamic, sequential EPC device (NormaTec Pro, Newton

171 Center, MA, USA) was applied for 30-min. Briefly, the EPC device utilizes "leg sleeves" which

172 contain five circumferential inflatable chambers (arranged linearly) encompassing the leg from

173 the feet to the hip/groin. The "leg sleeves" are connected to an automated pneumatic pump at

174 which duration of treatment and target inflation pressures can be controlled. In this study, we

175 chose to use target inflation pressures of ~70mmHg for each chamber as we've previously

176 observed acute improvements in vascular reactivity with bilateral treatment at this target inflation

177 pressure (Martin et al. 2015a). Herein, only one leg sleeve was applied (right leg) while the

178 contralateral leg (left) was uncovered. At the onset of the EPC treatment, all zones in the "leg

179 sleeve" are pre-inflated to form fit the participant's legs while applying minimal pressure (~10

180 mmHg). Thereafter, the distalmost chamber (which covers from the high ankle to toes) inflated

181 to approximately 70 mmHg while the remaining zones were not inflated. For 30-s, this chamber

182 "pulsed" after which the pressure was held constant at 70 mmHg and the same process occurred

183 in the zone above (calves) for another 30-s. Following this 30-s, the ankle zone deflated

184 completely, the calf remained at a constant pressure of approximately 70 mmHg, and the process

185 occurred in the next highest zone (high claves/lower thighs). This continued until the highest

186 zone (5th zone/upper thighs) finished the process at which point all zones were completely

187 deflated for 30-s and a full compression cycle (3-min total) was completed. This entire cycle of

188 compression was repeated continuously over the course of the treatment session which lasted for

189 30-min.

190 In AIM2, a sham condition was also employed which consisted of 30-min application of

191 a single EPC "leg sleeve" on the right leg, but devoid of any actual compression. A sham was

192   included in AIM2 to assess the thermogenic effect from contact and/or attenuation of heat loss

193   from simply wearing the "leg sleeve".

194   **Statistical Analysis**

195   All statistical analyses were performed using SPSS v22.0 (IBM Corp., Armonk, NY,

196   USA). Prior to statistical analysis normality was confirmed using Shapiro-Wilk tests. For

197   ANOVA, if sphericity was violated a correction factor was applied to hypothesis testing. Subject

198   characteristics for both aims were compared using independent t-tests.

199   For AIM1 dependent variables, two-way repeated measures ANOVAs were performed

200   with time (PRE vs. POST) and leg (treated vs. untreated) as independent variables. When a

201   significant time*leg interaction was observed, *post-hoc* comparisons were made using

202   Bonferroni corrected paired t-tests.

203   For AIM2, MST during treatment (i.e. T15) was analyzed using a repeated measures of

204   analysis with time (PRE vs. T15), region (thigh vs. lower-leg), and treatment group (EPC vs.

205   sham) as the independent variables. Given the opaque nature of the leg sleeve, leg (i.e., treated

206   vs. untreated) was not included as an independent variable as MST during respective treatments

207   was only evaluable in the untreated leg. MST at R0, R30 and R60 was analyzed using repeated

208   measures ANOVA with time (PRE vs. R0, R30 and R60), leg, region, and treatment group as the

209   independent variables. When significant interactions with time were found, *post-hoc*

210   comparisons were made using paired and independent t-tests as appropriate with Bonferroni

211   correction for multiple comparisons (0.05/number of comparisons). Data are presented as mean

212   ± standard deviation for summary data and as mean [95% confidence interval (lower limit, upper

213   limit)] for comparative data.

RESULTS

Subject Characteristics

Participant characteristics for the cohorts of subjects included in AIM1 and AIM2 are presented in Table 1. In AIM1, significant between sex differences existed for height, body mass, and blood pressures, but not age (p=0.211) and BMI (p=0.123). In AIM2, no significant between condition differences (i.e., EPC vs. sham) were found for any characteristic (p>0.05). Significant between sex differences existed for height (p=0.014), body mass (p=0.040), and diastolic blood pressure (p=0.039), but not systolic blood pressure (p=0.130), age (p=0.457) or BMI (p=0.203). However, sex was not found to be a significant covariate in either AIM1 or AIM2.

AIM1 - Effect of unilateral EPC on bilateral vascular reactivity in the legs.

Results from popliteal artery FMD assessment are presented in Table 2 and Fig. 2. No main effects (time or treatment) or interaction was observed for resting popliteal artery diameter or blood flow (Table 2; p>0.05 for all). With respect to the reactive hyperemia response, a significant main effect of time, but no main effect of leg or time*leg interaction, was observed for both maximum blood flow (Fig 2A) and shear rate AUC to peak dilation (p<0.010; Table 2). Independent of leg (collapsing across treated and untreated legs), maximum reactive hyperemia blood flow and shear rate AUC at the POST time point were decreased relative to baseline (i.e., PRE) by 39.3 mL/min [-0.9, -77.8] and 467 a.u. [-734, -200], respectively. No main effects or interactions were observed for total reactive hyperemia blood flow (Fig 2B).

A significant effect of time (p<0.001), but no main effect of leg or time*leg interaction, was observed for aFMD and pFMD. Independent of leg, both aFMD (+0.022 mm [+0.009, +0.035]; Table 2) and pFMD (+0.41% [+0.14, +0.68]; Fig. 2C) were significantly higher at the POST time point relative to PRE. For nFMD, significant main effects of time and leg were

238 observed, but no interaction (Fig. 2D). Independent of time (collapsing across PRE and POST

239 time points), nFMD was significantly higher in the treated leg (+0.31 [+0.10, +0.53]) and

240 independent of leg, nFMD was significantly higher at POST (+0.57 [+0.39, +0.80]).

241 **AIM2 - Effect of unilateral EPC on bilateral leg skin temperature**

242 Baseline (PRE) values for regional and average MST in each leg for each treatment group

243 are presented in Table 3. For during treatment (T15), significant time*treatment (p=0.003) and

244 time*region (p=0.031) interactions were observed. Across both regions, change in MST at T15

245 was significantly greater in the untreated leg with EPC compared to sham (+0.82 °C [+0.34,

246 +1.30]; Fig 3A). MST in the untreated leg was significantly elevated relative to baseline with

247 EPC treatment EPC and significantly reduced relative to baseline with sham in the untreated leg.

248 Across both treatments, change in MST was significantly greater in the thigh region compared to

249 the lower-leg region (+0.32 °C [+0.13, +0.51]; Fig 3B). However, collapsing across both

250 treatments, neither the thigh (p=0.051) or lower-leg (p=0.731) region were significantly different

251 from baseline.

252 For R0, significant region*time (p<0.001) and treatment*leg*time (p=0.032) interactions

253 were observed. Figure 3D illustrates the effect of region where, when collapsing across both

254 treatments and legs, MST was significantly elevated from baseline at R0 at both regions

255 (p<0.001 and p=0.003 for thigh and lower-leg, respectively) though the increase was

256 significantly greater in the thigh compared to the lower-leg (+0.55 °C [+0.40, +0.70]; p<0.001).

257 With respect to the treatment*leg*time interaction (illustrated in Fig. 3C), independent of region,

258 EPC treatment was associated with a significant increase in MST in both the treated leg

259 (p<0.001) and untreated leg (p=0.002) at R0 whereas MST was increased with sham in the

260 treated leg (p<0.001) but decreased in the untreated leg (p=0.002). Change in MST at R0 in the

261 treated leg with EPC treatment was significantly higher than in the untreated leg with EPC

262   treatment (+0.94 °C [+0.56, +1.31]; p<0.001) and the untreated leg with sham treatment (+1.81

263   °C [+1.24, +2.37]; p<0.001), but not the treated leg with sham treatment (+0.42 °C [-1.05,

264   +0.21]; p=0.076). Change in MST at R0 in the treated leg with sham was significantly higher

265   than in both the untreated leg with EPC (+0.52 [-0.03, +1.07]; p=0.012) and the untreated leg

266   with sham (+1.39 °C [+1.12, +1.65]; p<0.001). Finally, in the untreated leg, change in MST at

267   R0 was greater with EPC treatment compared to sham treatment (+0.87 °C [+0.35, +1.38];

268   p<0.001)

269   For the R30 time point, significant treatment*time (p<0.001), leg*time (p=0.001) and

270   region*time (p<0.001) interactions were observed. Across both regions and legs, MST was

271   significantly elevated relative to baseline with EPC treatment (p<0.001) and significantly

272   reduced relative to baseline with sham treatment (p=0.003; Fig. 4A). Across both treatments and

273   legs, thigh MST was significantly elevated relative to baseline (p<0.001), but not lower-leg MST

274   (p=0.558; Fig. 4B). Finally, across both regions and treatments, treated (p=0.034) and untreated

275   (p=0.873) leg MST were no longer significantly elevated relative to baseline (Fig. 4C).

276   Similar to the R30 time point, at the R60 time point, significant treatment*time

277   (p=0.002), leg*time (p=0.008) and region*time (p<0.001) interactions were observed. Across

278   both regions and legs, MST was no longer significantly elevated relative to baseline with EPC

279   treatment (p=0.051), but remained significantly reduced relative to baseline with sham treatment

280   (p<0.001; Fig. 4D. Across both treatments and legs, thigh MST was also no longer significantly

281   elevated relative to baseline (p=0.101), but now significantly reduced relative to baseline in the

282   lower-leg MST (p<0.001; Fig. 4E). Finally, across both regions and treatments, treated leg

283   (p=0.492) and untreated leg (p=0.075) MST remained unchanged relative to baseline (Fig. 4F).

Discussion

The primary findings of the present study are as follows; 1) 30-min of unilateral EPC is associated with increased popliteal artery FMD in both the treated and untreated leg post-EPC, 2) MST during treatment is significantly increased relative to baseline in the untreated leg with EPC treatment, but not sham treatment, 3) MST is increased in both treated and untreated legs immediately following EPC treatment, but only in the treated leg with sham treatment, 4) the increase in MST in the treated leg was more transient with sham treatment compared to EPC treatment, and 5) independent of other factors, the thigh region was associated with more robust and sustained increases in MST compared to the lower-leg.

Regarding AIM1 of the present investigation, the bilateral improvement in popliteal artery FMD with unilateral treatment is similar to our previous observations of improved popliteal (compressed leg) and brachial artery (non-compressed arm) FMD with 1-h of bilateral leg EPC treatment (Martin et al. 2015a). Herein, we extend our previous findings by demonstrating that 1) unilateral EPC improves vascular reactivity in the treated and untreated (contralateral) leg, 2) 30-min of treatment, compared to 1-h, is sufficient for improvement of peripheral conduit reactivity, and 3) FMD is improved for at least 30-min following treatment. Improved FMD within the treated leg could be explained by markedly altered hemodynamic shear stress, and resultant local nitric oxide (NO) bioavailability, during EPC treatment (Martin et al. 2016), but this is an unlikely mechanism in the contralateral leg. However, a single 1-h bout of IPC has been shown to affect systemic measures of NO as well as non-local vascular reactivity (Rifkind et al. 2014). While IPC does differ markedly from the EPC therapy used herein, it is not unreasonable to assume that other external compression stimuli can modulate changes in non-local and/or systemic NO bioavailability. We have also previously posited that

307   EPC-mediated myokine (e.g., IL-6) release may potentiate systemic NO bioavailability

308   (Fisslthaler & Fleming 2009; Horman et al. 2008; Pedersen & Febbraio 2008), but this remains

309   unknown. Moreover, while nitric oxide bioavailability may play a role in the observed acute

310   FMD increase in the untreated leg, a decrease in sympathetic nervous system (SNS) efferent

311   activity may also be involved in the observed response with EPC treatment (Dyson et al. 2006;

312   Hijmering et al. 2002; Lind et al. 2002). Indeed, the pressor effect from static compression on

313   the lower limb(s), at a range of pressures (30-90 mmHg), and with single limb compression, has

314   been shown previously and is abolished by epidural anesthesia (Williamson et al. 1994).

315   Moreover, we have previously observed acute increases in peripheral (brachial) and central

316   (aortic) blood pressure for at least 10-min following bilateral EPC treatment (Vincent-Horta et al.

317   2015). Thus, it is possible that the arterial baroreflex may modulate sympathetic outflow in

318   response to acute EPC treatment. However, the role of the sympathetic nervous system in the

319   acute FMD response to EPC requires further, specific, investigation.

320   In regards to AIM2 of the present investigation, thermography measures temperature

321   changes within the cutaneous microcirculation plexus that are modulated by skin blood flow

322   perfusion. As such, infrared imaging provides a non-contact thermal map of the skin surface

323   area that reveals both spatial and temporal changes that occur in the regional temperature

324   distribution (Pascoe et al. 2012). Notably, although infrared imaging provides accurate thermal

325   measures of skin surface temperatures that are responsive to blood flow alterations, it cannot

326   quantify blood flow itself.

327   In the treated legs, immediately following EPC/sham treatment (i.e., R0) a similar,

328   significant rise in temperature of greater than 1°C was observed with both EPC (+1.53 °C) and

329   sham (+1.12 °C). The EPC "leg sleeve" is a non-breathable, thick material for which conductive

330 contact did not allow for heat transfers (Havenith 1999), likely mediating a significant proportion

331 of the increase in MST of the treated leg with both EPC and sham. Indeed, while the change in

332 MST was greater in the treated leg with EPC compared to sham (+0.42) it was not a statistically

333 significant difference (p=0.073). However, in the untreated leg, immediately following

334 completion of sham/EPC treatment (i.e. R0), MST was significantly elevated relative to baseline

335 with EPC (+0.60 °C), but significantly decreased relative to baseline with sham (-0.27 °C)

336 illustrating a markedly different response to the two treatments. This was similar to what was

337 observed during treatment (i.e. T15), where there was a significant increase across both regions

338 in the untreated leg with EPC (+0.83 °C), but a significant decrease with sham (-0.20 °C). Thus,

339 it would appear that during and immediately following unilateral treatment, the addition of a

340 pneumatic compression stimulus not only prevents a decrease in MST in the contralateral limb,

341 but actually promotes a significant increase. Although skin sympathetic nerve activity (Wallin et

342 al. 1975) and sweat rate (Wilson et al. 2001) do not change with the aforementioned arterial

343 baroreflex, changes in cutaneous vascular conductance have been observed (Crandall et al. 1996;

344 Kellogg et al. 1990; Tripathi & Nadel 1986). Regardless, one explanation could simply be the

345 distribution, or re-distribution, of heat and/or blood in the vascular space (Arndt et al. 1985).

346 Indeed, the higher MST in the contralateral leg with EPC, but not sham, during and immediately

347 following treatment could be due to heat added to the system from the dynamic compression

348 stimulus (i.e., tissue deformation) and/or compression itself mobilizing fluid(s) including, but not

349 limited to, a proportion of venous and arterial flow.

350   Differential responses between treatments (EPC vs. sham) were also observed during the

351 recovery period from EPC/sham treatment (i.e., R30, R60). Indeed, 30-min following

352 completion of unilateral treatment, independent of leg, MST remained elevated respective to

353   baseline with EPC and was actually reduced relative to baseline with sham treatment. In

354   addition, 60-min following treatment, independent of leg, while MST had returned to baseline

355   with EPC treatment, it remained reduced relative to baseline with sham. The absence of a

356   significant interaction with the factor of leg (treated vs. untreated) suggests an additional effect

357   of unilateral dynamic compression on the bilateral thermal recovery response. In the treated leg,

358   transmural pressure changes in the vasculature of the compressed tissue are likely associated

359   with an auto-regulatory (i.e., myogenic) response which may augment skin blood flow and MST.

360   Importantly, this effect has been shown to persist for hours following pressure application

361   (Bochmann et al. 2005). However, devoid of a compression stimulus, this is unlikely to be a

362   major contributor to the contralateral limb. Thermal control over cutaneous vascular responses

363   has not been fully elucidated but have affirmed roles for temperature sensitive afferent neurons,

364   sympathetic/parasympathetic controls and NO mediated vasodilation (Kellogg 2006) which may

365   have, in some capacity, contributed to our observations. While speculative, acute massage, to

366   which lower limb EPC is often compared, has been shown to elicit a decrease in sympathetic

367   nervous system efferent activity and an increase in parasympathetic nervous system activity

368   (Diego & Field 2009). Moreover, twenty 40-min sessions of massage therapy over the course of

369   4-weeks has been shown to decrease autonomic nerve conduction latency and amplitude (Lee et

370   al. 2011). However, future studies are warranted with respect to autonomic nervous system

371   modulation and its role in local and non-local responses to EPC treatment.

372   Several findings herein would appear to be of interest from a clinical standpoint. First,

373   acute improvements in bilateral popliteal artery FMD would suggest improved peripheral

374   vascular reactivity which may potentiate greater blood flow and wound healing (Guo & Dipietro

375   2010). In addition, improved endothelium dependent and independent vascular reactivity may

376   decrease the risk for diabetic foot ulcer development (Dinh et al. 2012). Secondly, although we

377   did not observe any change following the EPC condition in peak or total reactive hyperemia

378   assessed with ultrasonography during the FMD procedure, this is similar to our previous findings

379   with EPC (Martin et al. 2015a). Notably, in that study, despite no changes in reactive hyperemia

380   assessed via ultrasonography, we did identify an acute increase in peak reactive hyperemia blood

381   flow responses immediately following an EPC condition when assessed via venous occlusion

382   plethysmography. Finally, local blood flow improvements may mitigate negative ischemic,

383   inflammatory, oxidative, and bacterial associated factors in wound healing (Guo & Dipietro

384   2010; Hopf et al. 1997; Kurz et al. 1996; Mustoe 2004). Herein, we observed marked increases

385   in MST, a surrogate of skin blood flow, in the contralateral leg during and for at least 30-min

386   following unilateral EPC treatment. Thus, non-contact treatment via compression of the

387   unaffected limb may be a viable strategy to improve skin blood flow during wound healing.

388   Importantly, at all time points (during and after treatment) significant effects of region were

389   observed with a more robust increase in thigh MST being observed initially with a subsequently

390   similar rate of decline over the recovery period. Thus, the regional nature of the responses needs

391   to be considered. In addition, from an experimental model perspective, our findings suggest

392   treatment differences may be disguised when using a one leg (control) model in which the

393   untreated leg is altered by the intervention. In our case, the increased temperatures in the

394   "control" leg lessened the calculated statistical effect between legs. Finally, we used target

395   inflation pressures of ~70 mmHg only for the EPC intervention and the response to higher or

396   lower target inflation pressure(s) may vary.

Conclusions

Unilateral, dynamic, whole-leg EPC treatment increases bilateral vascular reactivity and MST. With respect to MST, it is important to recognize that although there is a greater and more persistent effect in the treated leg with MST, single leg treatment will also provide a clinically significant increase in the contralateral limb. Moreover, EPC treatments of the leg were strongly influenced by the intervention response in the thigh, and to a lesser extent the lower leg. From a clinical perspective, our results suggest that EPC could be utilized to increase blood flow in an afflicted limb (e.g. injury, peripheral vascular disease, etc.) with contralateral application, particularly when the target area is more proximal in the leg.

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495

Participant characteristics for AIM1 and AIM2.

Participant characteristics for AIM1 and AIM2.

	AIM1			AIM2		
	Overall (N=18)	Males (n=9)	Females (n=9)	Overall (N=14)	EPC (n=7)	Sham (n=7)
Age, yrs	25.5 ± 4.7	26.9 ± 4.8	24.1 ± 4.3	25.9 ± 4.5	25.6 ± 4.0	26.2 ± 5.3
height, m	1.70 ± 0.09	1.76 ± 0.8	1.64 ± 0.05**	1.74 ± 0.09	1.75 ± 0.07	1.73 ± 0.10
body mass, kg	75.1 ± 16.0	84.4 ± 16.1	65.7 ± 9.6**	82.9 ± 14.9	85.8 ± 12.7	79.9 ± 17.3
BMI, kg/m^2	25.6 ± 3.5	26.9 ± 3.5	24.3 ± 3.2	27.2 ± 2.7	28.0 ± 2.4	26.4 ± 2.9
LA SBP, mmHg	112 ± 10	117 ± 9	108 ± 9*	117 ± 10	117 ± 10	117 ± 12
LA DBP, mmHg	71 ± 6	76 ± 4	66 ± 3**	74 ± 6	77 ± 3	72 ± 8
RA SBP, mmHg	111 ± 11	116 ± 10	105 ± 10*	115 ± 13	117 ± 11	114 ± 15
RA DBP, mmHg	71 ± 8	75 ± 7	66 ± 8*	74 ± 8	77 ± 5	71 ± 10

Data are mean ± SD. BMI, body mass index; LA, left arm; SBP, systolic blood pressure; DBP, diastolic blood pressure; RA, right arm. **, * $p<0.05$ and $p<0.01$, respectively, for between sex comparisons (AIM1)

Popliteal artery flow-mediated dilation characteristics at baseline and after EPC in the treated and non-treated leg.

Popliteal artery flow-mediated dilation characteristics at baseline and after EPC in the treated and non-treated leg

	EPC (n=18)	
	PRE	POST
Treated Popliteal Artery		
Resting diameter, mm	5.41 ± 0.96	5.49 ± 0.94
Resting MBV, cm/s	7.7 ± 1.9	7.4 ± 1.9
Resting mean shear rate, s^{-1}	58.2 ± 14.0	55.3 ± 15.2
Reactive hyperemia MBV AUC, cm/s	300 ± 119	$243 \pm 121*$
Reactive hyperemia shear rate AUC, A.U.	2245 ± 897	$1878 \pm 1204*$
Absolute FMD, mm	0.11 ± 0.04	$0.13 \pm 0.05*$
Untreated Popliteal Artery		
Resting diameter, mm	$5.12 \pm 0.92†$	$5.16 \pm 0.86†$
Resting MBV, cm/s	8.0 ± 1.2	$7.1 \pm 1.6*$
Resting mean shear rate, s^{-1}	64.6 ± 14.9	$56.2 \pm 13.8*$
Reactive hyperemia MBV AUC, cm/s	331 ± 129	$266 \pm 121*$
Reactive hyperemia shear rate AUC, A.U.	2688 ± 1147	$2121 \pm 1026*$
Absolute FMD, mm	0.09 ± 0.03	$0.12 \pm 0.04*$

Data are expressed as mean ± SD. Mean blood velocity (MBV) and shear rate were measured continuously and resting measures are representative of the average of 2 minutes of baseline measurements, whereas area under the curve (AUC) measures are representative of values following cuff release until maximal dilation was observed ; FMD is flow mediated dilation expressed as absolute (mm), relative (%), and normalized to the reactive hyperemic shear rate AUC. * and **, significantly difference between time points (pre vs. post; ($P<0.05$ and $P<0.01$, respectively); †, significantly different between legs at the same time point (e.g. post in compressed artery vs. post in non-compressed artery; $P<0.05$)

Average and regional mean skin temperature (MST) at baseline in each leg.

Average and regional mean skin temperature (MST) at baseline in each leg

	Right leg			Left Leg		
	Thigh MST	*Lower-leg MST*	*Average MST*	*Thigh MST*	*Lower-leg MST*	*Average MST*
EPC (n=7)	30.6 ± 1.1	31.1 ± 0.8	30.9 ± 1.0	31.0 ± 0.9	31.0 ± 1.1	31.0 ± 1.0
Sham (n=7)	30.6 ± 0.5	31.2 ± 0.7	30.9 ± 0.6	30.5 ± 0.5	31.2 ± 0.7	30.9 ± 0.6

Data are mean ± SD. EPC, external pneumatic compression.

Figure 1

Representative thermograms.

Pictured are thermograms of a participant's lower limbs (A) after equilibration to environmental conditions (i.e. baseline [PRE]), (B) during external pneumatic compression (EPC; T15), and (C) immediately post-EPC (R0).
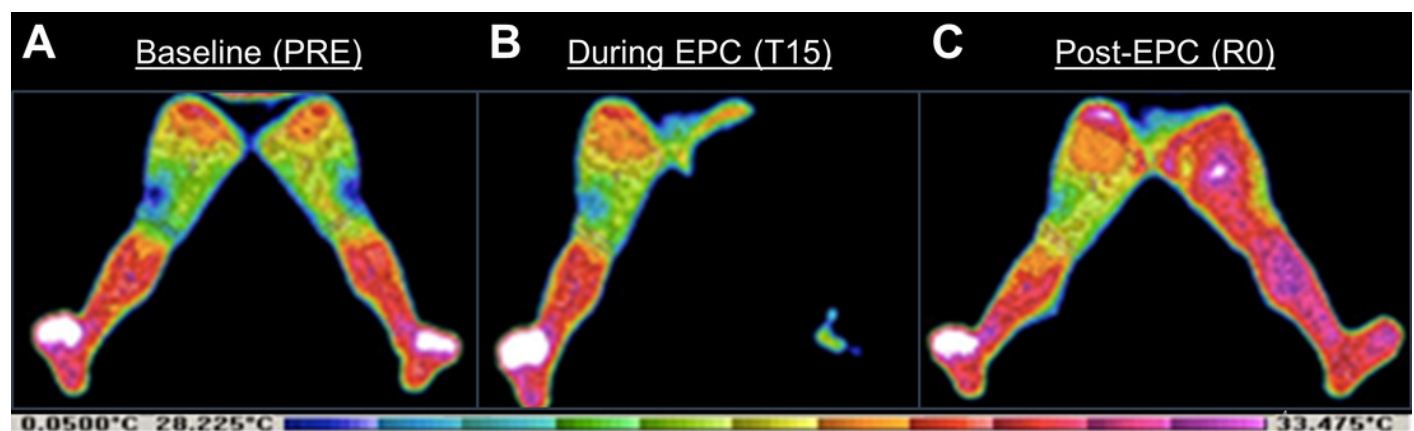
Figure 2

Popliteal artery blood flow and flow mediated dilation (FMD) characteristics in both legs before (PRE) and 30-min after (POST) a bout of unilateral external pneumatic compression (EPC).

A) maximal (max) popliteal artery blood flow during reactive hyperemia, B) total popliteal artery blood flow during reactive hyperemia, C) relative FMD (%), and D) FMD normalized to shear rate area under the curve to peak dilation (nFMD). Data are presented as mean ± SD for each leg (treated vs. untreated) at each time point (PRE vs. POST). ANOVA results are presented within each panel with significant main effects emphasized in bold.
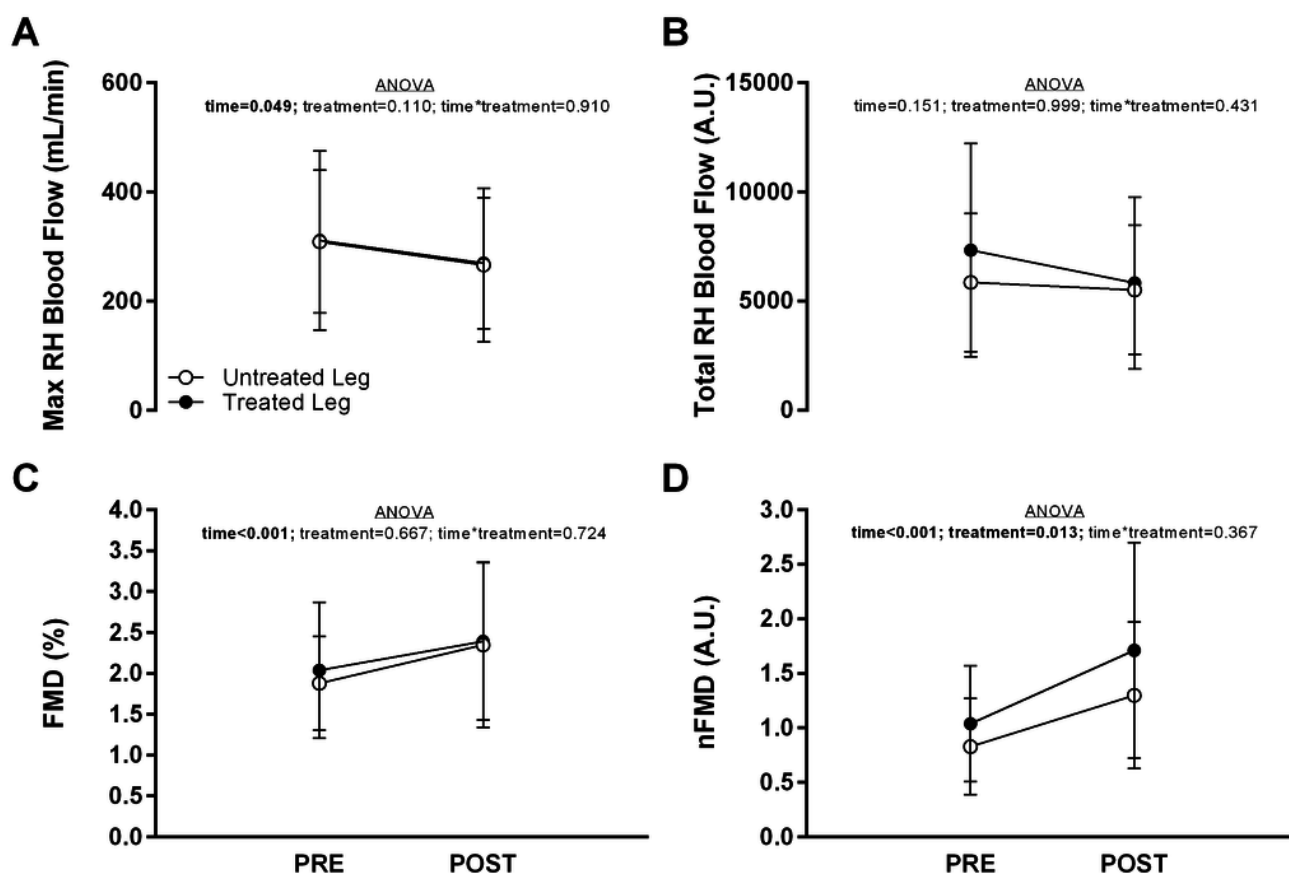
Figure 3

Change (Δ) from baseline in mean skin temperature (MST) at the mid-point (T15) and immediately following (R0) external pneumatic compression (EPC)/sham treatment.

Data are presented as individual values and mean ± 95% confidence interval. Panels illustrate the observed A) time*treatment (EPC vs. Sham; data collapsed across regions) and B) time*region (thigh vs. lower-leg; data collapsed across treatments) interactions for MST at T15, and C) time*treatment*leg (treated vs. untreated; data collapsed across regions) and D) time*region (data collapsed across legs and treatments) interactions for MST at R0. *, significant change from baseline (p<0.0167) and ϕ, significantly different between factors (p<0.0167). For panel c: †, significant change from baseline (p<0.0125) and different superscript letters between groups indicate significant differences (p<0.008).
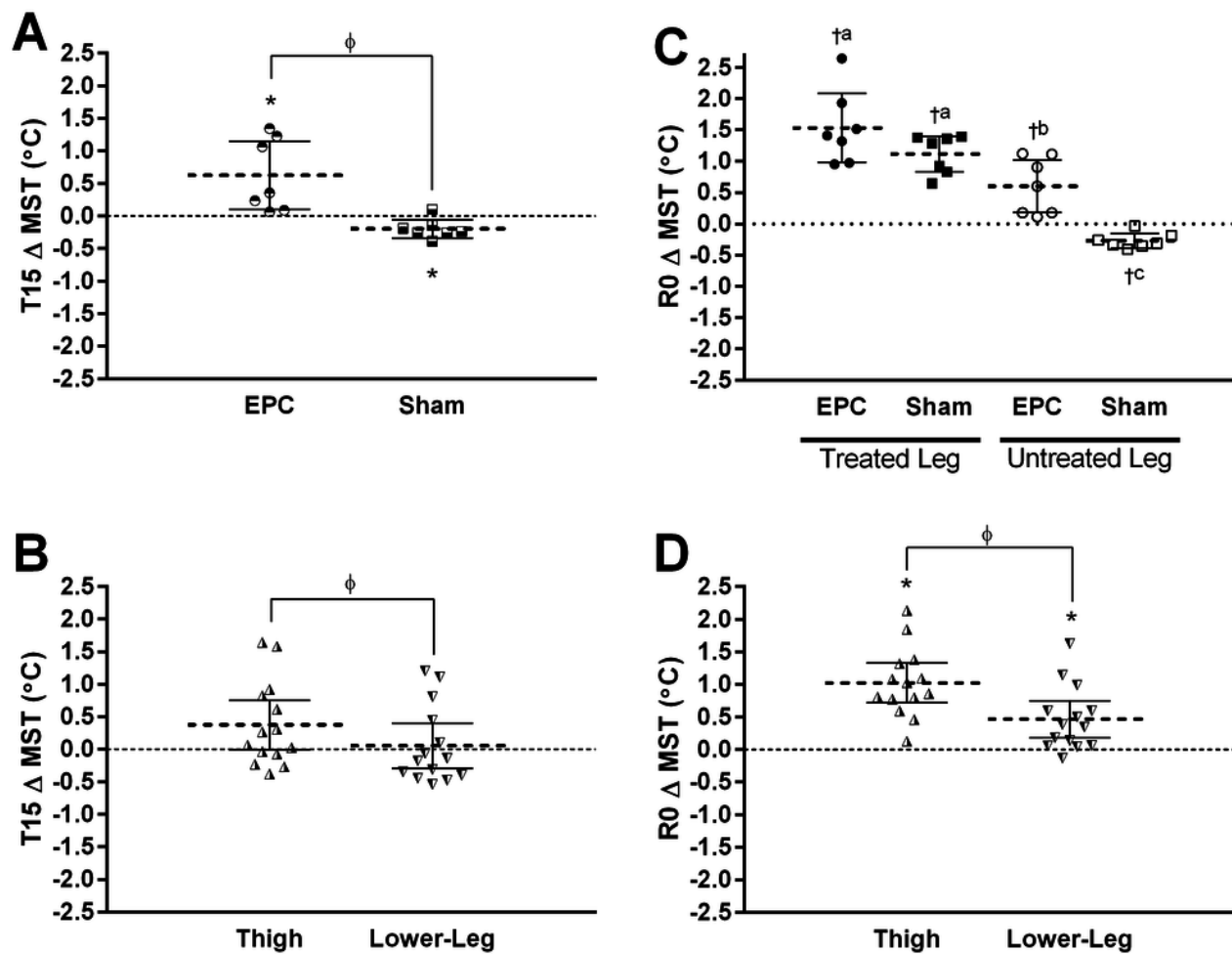
Figure 4

Change from baseline in mean skin temperature (MST) at 30- (R30) and 60-min (R60) following external pneumatic compression (EPC)/sham treatment.

Data are presented as individual values and mean ± 95% confidence interval. Panels illustrate the observed A) time*treatment (EPC vs. Sham; data collapsed across regions and legs), B) time*region (thigh vs. lower-leg; data collapsed across treatments and legs), and C) time*leg (treated vs. untreated; data collapsed across treatments and regions) interactions for MST at R30 and D) time*treatment, E) time*region, and F) time*leg interactions for MST at R30. *, significant change from baseline (p<0.0167) and φ, significantly different between factors (p<0.0167).