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SHORT REPORT

Vascular Biology and Microcirculation

Remote and local effects of ischemic preconditioning on vascular function: a case for cumulative benefit

Bert Bond, Harrison Hurlstone, David M. Köditz, Alice B. Lester, Harry Mould, Thomas Tennant, and Amber Thorington

Public Health and Sport Sciences, University of Exeter, Devon, United Kingdom

Abstract

Brief, repeated cycles of limb ischemia and reperfusion [ischemic preconditioning (IPC)] can protect against vascular insult. Few papers have considered the effect of IPC on resting vascular function, and no single study has simultaneously considered the local (trained arm) and remote (untrained arm) effects of a single session of IPC and following repeated sessions. We determined macrovascular [allometrically scaled flow-mediated dilation (FMD)] and microvascular [cutaneous vascular conductance (CVC)] function in healthy adults before, immediately post, 20 min post, and 24 h post a single session of IPC (4×5 min of single arm ischemia). These outcomes also were remeasured 24 h after six IPC sessions, performed over 2 wk. FMD and CVC increased in both arms 20 min post [FMD mean difference (MD) 1.1%, $P < 0.001$; CVC MD 0.08 arbitrary units (AU), $P = 0.004$] but not 24 h post (FMD MD −0.2%, $P = 0.459$; CVC MD −0.02 AU, $P = 0.526$] a single session of IPC, with no differences between trained and untrained arms. Although FMD did not increase 24 h after one IPC session, it was elevated in both arms 24 h after the sixth session (MD 1.2%, $P = 0.009$). CVC was not altered in either arm 24 h after the last IPC session. These data indicate that the local and remote effects of IPC on vascular health may be equivalent and that the benefits to FMD may be greater with sustained training compared with a single IPC exposure.

NEW & NOTEWORTHY For the first time in a single study, we demonstrate that resting macro- and microvascular function increases 20 min, but not 24 h, after a single IPC session and that the magnitude of this improvement was the same in the trained arm and nontrained, contralateral arm. In contrast, macrovascular (but not microvascular) function was augmented 24 h after six IPC training sessions in both arms, suggesting a cumulative benefit of IPC training on macrovascular function.

blood flow restriction; endothelial function; flow-mediated dilation; microvascular function; occlusion

INTRODUCTION

Ischemic preconditioning (IPC) is a technique whereby tissues are deliberately exposed to repeated cycles of blood flow restriction and subsequent reperfusion for local (1) and remote (2) benefits. This includes indices of vascular function, even in ostensibly healthy individuals. However, most studies consider the influence of IPC on the protection from vascular insult—for example, ischemic-reperfusion injury (3). Although this may offer some clinical context, there is a paucity of data regarding how a single session of IPC might influence vascular function at rest. Encouragingly, a single session of IPC has been shown to acutely improve resting endothelial function, as assessed by flow-mediated dilation (FMD) in healthy young and older adults (4). However, FMD was only assessed immediately after the last ischemic exposure. This is limited given that a distinct time course in the FMD response has been observed elsewhere (3). A single IPC session has also been shown to promote microvascular reactivity in the contralateral limb 48 h later (5). However, these authors did not assess the more immediate (<1 h) microvascular response.

Regarding the influence of repeated IPC on vascular function, Jones et al. (6) demonstrated that 7 days of IPC training improved FMD and cutaneous vascular conductance (CVC) in both the trained and untrained limb. Interestingly, the magnitude of these improvements in the contralateral limb was approximately half that of the trained arm. A further study by this group reported that three such IPC sessions per week for 2 wk improved FMD, but not CVC, in the trained arm, although the contralateral limb was not assessed (7).

Presently, no within-measures study has simultaneously assessed the magnitude of any local and remote macro- and microvascular responses to a single session of IPC. Furthermore, it is unknown whether this response is

Correspondence: B. Bond (b.bond@exeter.ac.uk).
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different from any changes observed after repeated IPC exposure. This is necessary to understand whether the benefit of IPC training is cumulative, that is, more than any repeated "acute" effect of a single IPC session. The purpose of this study was to address this research gap and better characterize the macro- and microvascular responses to IPC.

MATERIALS AND METHODS

Following ethical approval, 17 ostensibly healthy young adults (8 females, 23.6 ± 4.1 yr, body mass index 24.8 ± 2.8 kg/m^2) provided written, informed consent to participate. Exclusion criteria included known cardiovascular disease or the use of any medication or supplement that might influence blood pressure or vascular function.

Study Design

Participants completed four separate visits to the laboratory as described in Fig. 1, to establish the influence of a single IPC session (*visits 2* and *3*) and 2 wk of IPC training (*visits 1–4*). The IPC stimulus was consistently delivered to a single arm only, with the other serving as an untrained control arm. Nine participants received the IPC training in their dominant arm, whereas eight participants trained their nondominant arm.

All measures occurred at 08:00 or 09:30 h (consistent within a participant) in a quiet, darkened, temperature-controlled (23°C) room. Participants reported to the laboratory following an overnight fast, including caffeine abstention, and having avoided strenuous exercise for 24 h. Blood pressure was measured at least twice, in the supine position, using an automated device (Dinamap V100; GE Healthcare) before the assessment of vascular function. Apart from the IPC training, participants were instructed to maintain their daily habits throughout the study period.

Influence of a Single Session of IPC Training

The effect of a single IPC session was considered by assessing the vascular response to the first IPC training session (*visits 2* and *3*, as described in Fig. 1). Macro- and microvascular function was first simultaneously assessed in both arms by considering the response to a 5-min period of ischemia. Participants then received two further 5-min periods of ischemia in the target arm only. Both arms then received the "fourth" ischemic period, and the vascular responses were noted ("immediately post-IPC"). Vascular function was then reassessed in both arms 20 min later ("20 min post"). To capture any "late effect" of IPC (3), vascular function was simultaneously reassessed in both arms 24 h later ("24 h post").

Influence of 2 wk of IPC Training

Macro- and microvascular function were simultaneously assessed in both arms before (*visit 1* "preliminary visit") and after (*visit 2* "pretraining") an initial 2-wk control period (Fig. 1). *Visit 2* allowed for the first IPC session to be performed under supervision, and for the acute vascular response to be scrutinized, as detailed earlier. IPC training always consisted of four repeated periods of forearm cuff occlusion on one arm only, each 5 min in length, and separated by 5 min. After the first IPC session (*visit 2*), participants were supplied with a sphygmomanometer (Welch Allyn DS54) and performed five further IPC training sessions at home. Participants were instructed to complete three IPC sessions each week, with the final IPC session performed 24 h before returning to the laboratory (*visit 4* "24-h post-*session 6*").

Macrovascular Function

Macrovascular function was assessed via FMD, in line with current guidelines (8). Briefly, the brachial artery was

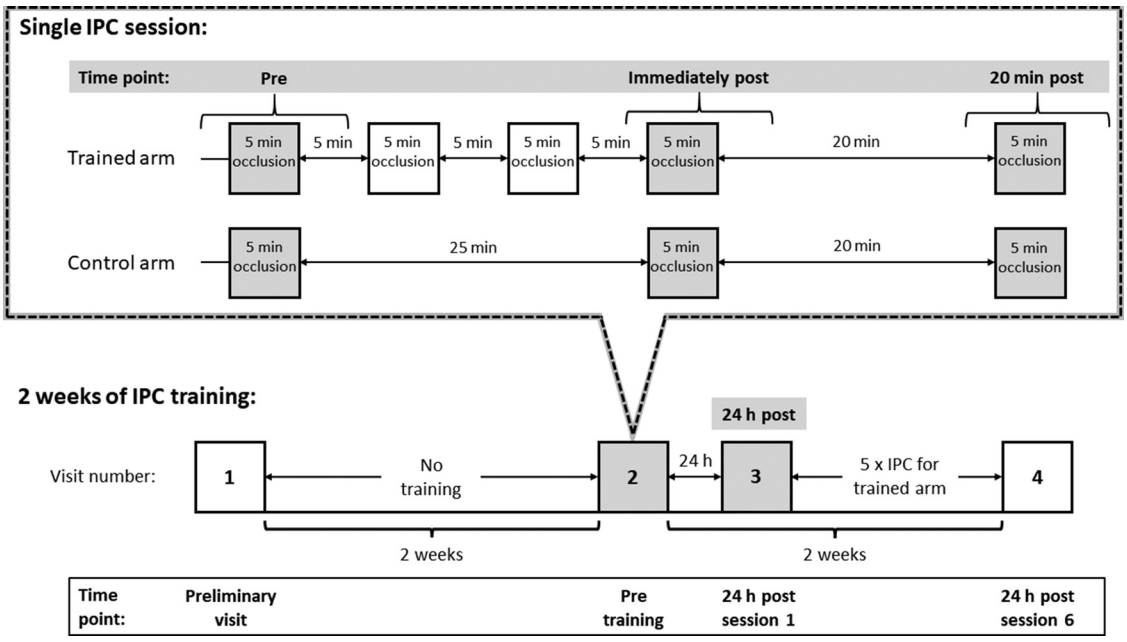

Figure 1. Protocol schematic. Each ischemic preconditioning (IPC) training session consisted of four periods of 5 min forearm occlusion, each separated by 5 min. Data used to consider the effect of a single IPC session are depicted in gray.

imaged in the distal third of the upper arm using high-resolution duplex ultrasonography (left arm: Apogee 1000, SIUI, China; right arm: Sequoia 512, Acuson). Arterial diameter was continually assessed for 1 min before and 3 min after 5 min of ischemia induced by rapid forearm cuff inflation (Hokanson) to 220 mmHg. Arterial diameter was assessed during end-diastole (Medical Imaging Applications LLC), by a single researcher who was blinded to the intervention. The FMD statistic was calculated as the percent increase in arterial diameter above baseline. To address concerns about the ratio-scaled FMD statistic, the vasodilatory response was also allometrically scaled via log-linear regression (9). Finally, the area under the curve for shear rate (SR_{AUC}) was calculated from the point of cuff deflation until the time of peak vasodilation (10). FMD was not normalized to SR_{AUC} as these outcomes were not consistently related. The between-day coefficient of variation (calculated from *visits 1* and *2*) for FMD of the IPC-trained arm and contralateral, nontrained control arm were 4.9 and 7.4%, respectively.

Microvascular Function

Postocclusive reactive hyperemia was assessed during the FMD protocol via laser Doppler perfusion monitoring (Moor Instruments Ltd., UK) (11). A low-powered probe (785 nm at 2.5 mW) was affixed to the distal third of the forearm. CVC was calculated as the average baseline flux divided by mean arterial pressure (MAP). Peak reactive hyperemia (PRH) was calculated as the maximal increase in flux following cuff release as a percentage of preocclusion baseline. The total reactive hyperemic response was defined as the incremental area under the postocclusive hyperemic curve adjusted to the postdeflation plateau (12). The between-day coefficient of variation for microvascular outcomes of the IPC-trained arm and contralateral, nontrained control arm were CVC 31.6 and 25.8%, PRH 31.7 and 34.1%, and total hyperemic response 28.0 and 38.2%.

Statistical Analyses

Macro- and microvascular outcomes were analyzed using a mixed-model ANOVA, with arm (IPC or nontrained control) and time point as the main effects. To consider the effect of a single IPC training session, the time points were all taken from laboratory *visits 2* and *3*, as depicted in gray in Fig. 1. The influence of 2 wk of IPC was analyzed using each of the four laboratory visits. For allometrically adjusted FMD, brachial diameter changes on the logged scale were analyzed with adjustment for the logarithmically transformed baseline diameter (9). Differences on the log scale were then back transformed to provide a percent diameter change. Point estimates are presented together, with 95% confidence intervals (95% CIs) of the mean difference (MD). There was no interaction effect of sex for any outcome, so data were pooled for men and women.

Effect sizes for the ANOVA main and interaction effects (η_P^2) were interpreted as small <0.06, moderate 0.06–0.14, and large >0.14. Follow-up pairwise comparisons were interpreted using 95% confidence intervals (95% CIs) of the mean difference (MD) and the *P* value. All data are presented as means ± SD.

RESULTS

One individual was removed from FMD analyses because of poor quality ultrasound images. In addition, one participant was unable to complete the 2-wk IPC training period because of unrelated circumstances.

MAP was never different at any time point (preliminary visit, 82 ± 6 mmHg; pre-IPC, 82 ± 7 mmHg; 24-h post-first IPC session, 81 ± 7 mmHg; 24-h post-sixth IPC session, 83 ± 10 mmHg; $P = 0.372$, $\eta_P^2 = 0.082$).

Influence of a Single Session of IPC

Macrovascular function.

Macrovascular outcomes are presented in Fig. 2. There was no significant effect of arm, time, or their interaction for baseline diameter or SR_{AUC}. However, there was a significant main effect for time ($P < 0.001$, $\eta_P^2 = 0.506$) for ratio-scaled FMD. When compared with pre-IPC, FMD in both arms was lower immediately after the IPC session (MD −0.9%, 95% CI −1.5 to −1.3, $P = 0.004$), augmented 20 min post (MD 1.1%, 95% CI 0.6–1.6, $P < 0.001$) but not different to baseline 24 h post (MD −0.2%, 95% CI −0.6 to 0.3, $P = 0.459$). There were no differences in FMD responses between the IPC-trained or nontrained contralateral arm (main effect of arm $P = 0.967$, $\eta_P^2 < 0.001$; arm by time interaction $P = 0.776$, $\eta_P^2 = 0.024$).

Allometrically adjusted FMD replicated the pattern observed with the ratio-scaled statistic. There was a significant effect of time ($P = 0.018$, $\eta_P^2 = 0.596$), but there were no differences in responses between the IPC-trained or nontrained contralateral arm (main effect of arm $P = 0.463$, $\eta_P^2 = 0.033$; arm by time interaction $P = 0.971$, $\eta_P^2 = 0.015$). When compared with pre-IPC, FMD in both arms was lower immediately after the IPC session (MD −0.9%, 95% CI −1.5 to −1.3, $P = 0.004$), augmented 20 min post (MD 1.1%, 95% CI 0.6–1.6, $P < 0.001$) but not different to baseline 24 h post (MD −0.2%, 95% CI −0.6 to 0.3, $P = 0.459$).

Microvascular function.

Microvascular responses to the first IPC session are presented in Table 1. A significant main effect of time ($P = 0.045$, $\eta_P^2 = 0.212$), but not arm ($P = 0.216$, $\eta_P^2 = 0.100$) or arm by time interaction ($P = 0.218$, $\eta_P^2 = 0.099$), was present for resting CVC. When compared with pre-IPC, CVC was greater in both arms immediately post [MD 0.08 arbitrary units (AU), 95% CI 0.05–0.12, $P < 0.001$] and 20 min post-IPC (MD 0.08 AU, 95% CI 0.03–0.13, $P = 0.004$), but not 24 h post (MD −0.02 AU, 95% CI −0.08 to 0.04, $P = 0.526$). There was no main effect of arm, time, or their interaction for PRH ($P > 0.800$, $\eta_P^2 < 0.022$ for all) or the total hyperemic response ($P > 0.087$ and $\eta_P^2 < 0.183$ for all).

Influence of 2 wk of IPC Training

Macrovascular function.

The macrovascular responses to six sessions of IPC are presented in Fig. 3. There was no significant effect of arm, time, or their interaction for baseline diameter or SR_{AUC}. A significant main effect of time ($P = 0.005$, $\eta_P^2 = 0.334$) was observed for ratio-scaled FMD, but not arm ($P = 0.613$, $\eta_P^2 = 0.019$) or time by arm interaction ($P = 0.374$, $\eta_P^2 = 0.071$). FMD in both arms was greater 24 h after the 2-wk training

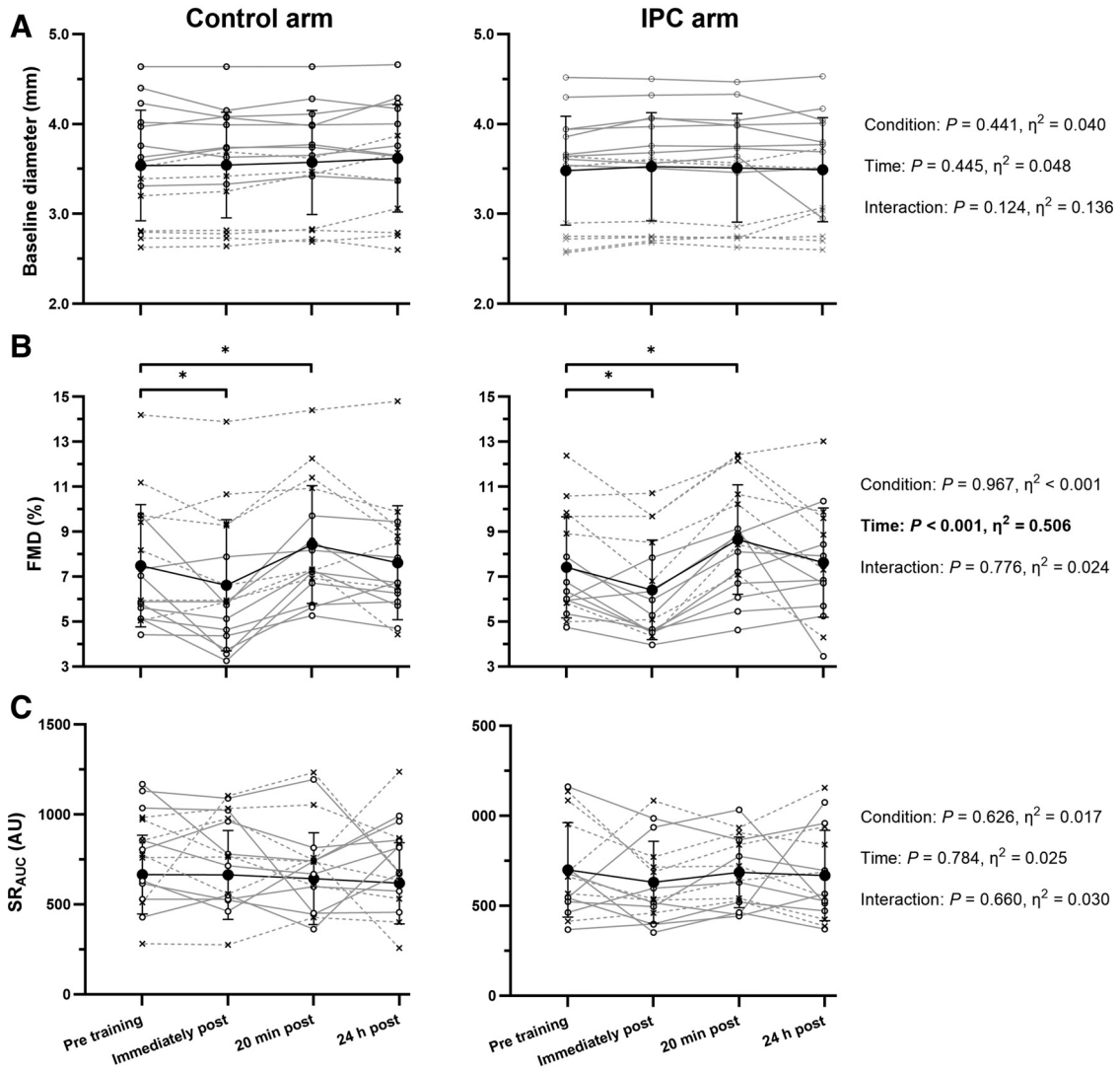

Figure 2. Macrovascular responses to a single ischemic preconditioning (IPC) session. *A*–*C*: baseline diameter (*A*), flow-mediated dilation (FMD; *B*), and area under the curve for shear rate (SR_{AUC}; *C*). Data are presented for men ("o" and sold line) and women ("x" and dashed line) with means and SD in black.

period (*visit 4*) compared with the preliminary visit (*visit 1*; MD 1.0%, 95% CI 0.5–1.6, $P = 0.004$). FMD was also greater after training compared with the pretraining time point (*visit 2*; MD 0.9%, 95% CI 0.4–1.5, $P = 0.002$). The difference in FMD 24 h after the 2-wk training period compared with 24 h after the first IPC session did not achieve statistical significance (*visit 4* vs. *visit 3*; MD 0.7%, 95% CI −0.1 to 1.5, $P = 0.070$).

Allometrically scaled FMD echoed the pattern observed with the ratio-scaled FMD outcome. Specifically, there was a significant effect of time ($P = 0.018$, $\eta_P^2 = 0.412$), but not arm ($P = 0.324$, $\eta_P^2 = 0.062$) or arm by time interaction ($P = 0.999$, $\eta_P^2 = 0.002$). Allometrically adjusted FMD was greater in both arms 24 h after the 2 wk of training (*visit 4*) compared with the preliminary visit (*visit 1*; MD 1.3%, 95% CI 0.4–2.2, $P = 0.004$) and pretraining time point (*visit 2*; MD 1.2%, 95% CI 0.3–2.1, $P = 0.009$). The difference between 24 h post-2 wk of training and 24 h after the first IPC session failed to reach statistical difference (*visit 4* vs. *visit* 3; MD 0.8%, 95% CI −0.1 to 1.7, $P = 0.066$).

Microvascular function.

The microvascular responses to six sessions of IPC are presented in Table 1. No significant main or interaction effects were present for resting CVC ($P > 0.085$, $\eta_P^2 < 0.166$) or PRH ($P > 0.251$, $\eta_P^2 < 0.106$ for all). There was no significant effect of time ($P = 0.249$ and $\eta_P^2 = 0.110$) or arm by time interaction ($P = 0.705$ and $\eta_P^2 = 0.038$) for the total hyperemic response, but there was a main effect of arm ($P = 0.043$ and $\eta_P^2 = 0.299$). When averaged across all time points, the total hyperemic response was greater in the arm that received the IPC training (MD 870 AU, 95% CI 33–1,708, $P = 0.043$). However, differences between arms at each time point never achieved statistical significance ($P > 0.081$ for all comparisons).

DISCUSSION

We observed that *1*) FMD and CVC were augmented acutely (20 min) after a single session of IPC; however, this improvement was lost 24 h later; *2*) we noted that the magnitude of this improvement was similar between the limb

Table 1. *Microvascular responses to a single session and 2 wk (6 sessions) of IPC*

	Time Point				ANOVA Effects		
	Pretraining	Immediately post	20-min post	24 h post	Arm	Time	Arm by time
Single session of IPC							
Resting CVC, AU							
Trained	0.31 ± 0.13	0.44 ± 0.22*	0.42 ± 0.23*	0.32 ± 0.18	$P = 0.216$	$\mathbf{P = 0.045}$	$P = 0.218$
Control	0.28 ± 0.16	0.32 ± 0.21*	0.32 ± 0.22*	0.31 ± 0.15	$\eta_p^2 = 0.100$	$\mathbf{\eta_p^2 = 0.212}$	$\eta_p^2 = 0.099$
PRH, %							
Trained	593 ± 197	554 ± 201	587 ± 269	598 ± 157	$P = 0.879$	$P = 0.853$	$P = 0.800$
Control	598 ± 166	568 ± 205	563 ± 219	571 ± 227	$\eta_p^2 = 0.002$	$\eta_p^2 = 0.017$	$\eta_p^2 = 0.022$
Total hyperemic response, AU							
Trained	6,660 ± 3,787	7,102 ± 3,733	7,465 ± 4,113	7,283 ± 4,392	$P = 0.087$	$P = 0.905$	$P = 0.326$
Control	6,184 ± 3,704	5,607 ± 4,128	5,736 ± 3,990	5,794 ± 3,428	$\eta_p^2 = 0.183$	$\eta_p^2 = 0.003$	$\eta_p^2 = 0.069$
	Preliminary visit	**Pretraining**	**24-h post-*session 1***	**24-h post-*session 6***			
Two weeks of IPC							
Resting CVC, AU							
Trained	0.25 ± 0.15	0.29 ± 0.11	0.32 ± 0.19	0.34 ± 0.16	$P = 0.334$	$P = 0.085$	$P = 0.817$
Control	0.21 ± 0.09	0.28 ± 0.18	0.31 ± 0.16	0.29 ± 0.20	$\eta_p^2 = 0.078$	$\eta_p^2 = 0.166$	$\eta_p^2 = 0.025$
PRH, %							
Trained	686 ± 303	632 ± 193	615 ± 113	534 ± 110	$P = 0.750$	$P = 0.251$	$P = 0.563$
Control	635 ± 163	612 ± 183	586 ± 245	583 ± 208	$\eta_p^2 = 0.009$	$\eta_p^2 = 0.106$	$\eta_p^2 = 0.045$
Total hyperemic response, AU							
Trained	5,831 ± 2,962	6,200 ± 2,833	7,791 ± 4,595	6,752 ± 3,245	$\mathbf{P = 0.043}$	$P = 0.249$	$P = 0.705$
Control	5,384 ± 2,717	5,904 ± 3,259	6,453 ± 3,132	5,353 ± 2,133	$\mathbf{\eta_p^2 = 0.299}$	$\eta_p^2 = 0.110$	$\eta_p^2 = 0.038$

Values are means ± SD CVC, cutaneous vascular conductance; PRH, peak reactive hyperemia. When considering the effect of a single ischemic preconditioning (IPC) session, repeated-measures ANOVA revealed a main effect of time for CVC only. *Follow-up pairwise comparisons revealed significant within-arm differences to pre-IPC only. Regarding 2 wk of IPC training, repeated-measures ANOVA revealed a significant main effect of arm for the total hyperemic response (shown in boldface); however, follow-up pairwise comparisons failed to reveal any significant between-arm differences at any time point ($P > 0.081$ for all).

exposed to the IPC stimulus and the contralateral, untrained limb; *3*) despite no change in vascular function 24 h after a single IPC session, improvements in FMD (but not CVC) were apparent 24 h after the sixth session; and *4*) the magnitude of this improvement was again similar between trained and untrained arms.

The improvement in resting FMD after 2 wk (6 sessions) of IPC agrees with existing data in healthy adults (7). However, a point of difference in our study was the simultaneous assessment of the nontrained contralateral arm. We report here that this remote effect of IPC was of a similar magnitude, which indicates a wider utility of IPC. In contrast, another study by this group reported a greater FMD increase in the trained compared with the nontrained arm after 7 days of daily IPC, although such difference did not achieve statistical significance (6). This small disparity in findings might be due to the frequency of the IPC training sessions (7). Little is known about how an IPC protocol can be optimized, either as a single session or when repeatedly delivered. This remains a pertinent research question given the encouraging observations here.

An important, novel finding in our study was that FMD was unaltered in either arm 24-h post-IPC but was improved 24 h the final (6th) training session. This suggests that a cumulative influence of IPC training is present, which is beyond that of any repeated "acute" effect from the last IPC session. This tallies with observations that the benefit of seven daily IPC sessions might still be detected 8 days later (6). However, the lack of improvement in resting FMD 24 h post a single IPC session might not mean the absence of any vascular benefit. It is possible that this single session may have afforded some protection against a vascular challenge, which has been observed using ischemic-reperfusion injury models (3), but this was beyond the scope of our study.

We observed changes in resting CVC in the immediate aftermath of IPC, but this change was lost 24 h the first and last IPC session. Improvements in resting CVC have been observed after IPC training (6), although this may be dependent on the frequency of training sessions (7). In this manner, our failure to observe improvements in resting CVC after six IPC sessions delivered over 2 wk is consistent with existing data.

This study is the first to consider the local and remote, macro- and microvascular responses to a single session of IPC and also IPC training. The within-measures study design, application of the latest FMD guidelines (8), and replication of existing IPC protocols (5–7) to facilitate cross-study comparisons are strengths of this work. However, we acknowledge several limitations. First, our study is unable to provide any mechanistic insight, beyond the understanding that the FMD outcome, but not the cutaneous reactive hyperemic response, is nitric oxide dependent (12, 13). A plethora of different mechanisms have been argued to play a role (14), and their contribution may differ regarding the "early" (<2 h) and "late" (>24 h) effects of IPC (15). However, understanding such processes was not the purpose of this study. We were also unable to consider other parameters of vascular health, such as endothelial-independent alterations in arterial function or indices of stiffness. Second, our study design did not include a "detraining" period. This would have provided insight into the legacy of the 2-wk IPC training intervention, which may feasibly last several days (5, 6). It is also feasible that alterations in vascular function might be observed 48 h, but not 24 h following a single IPC exposure, which we may have missed (5). Finally, we are unable

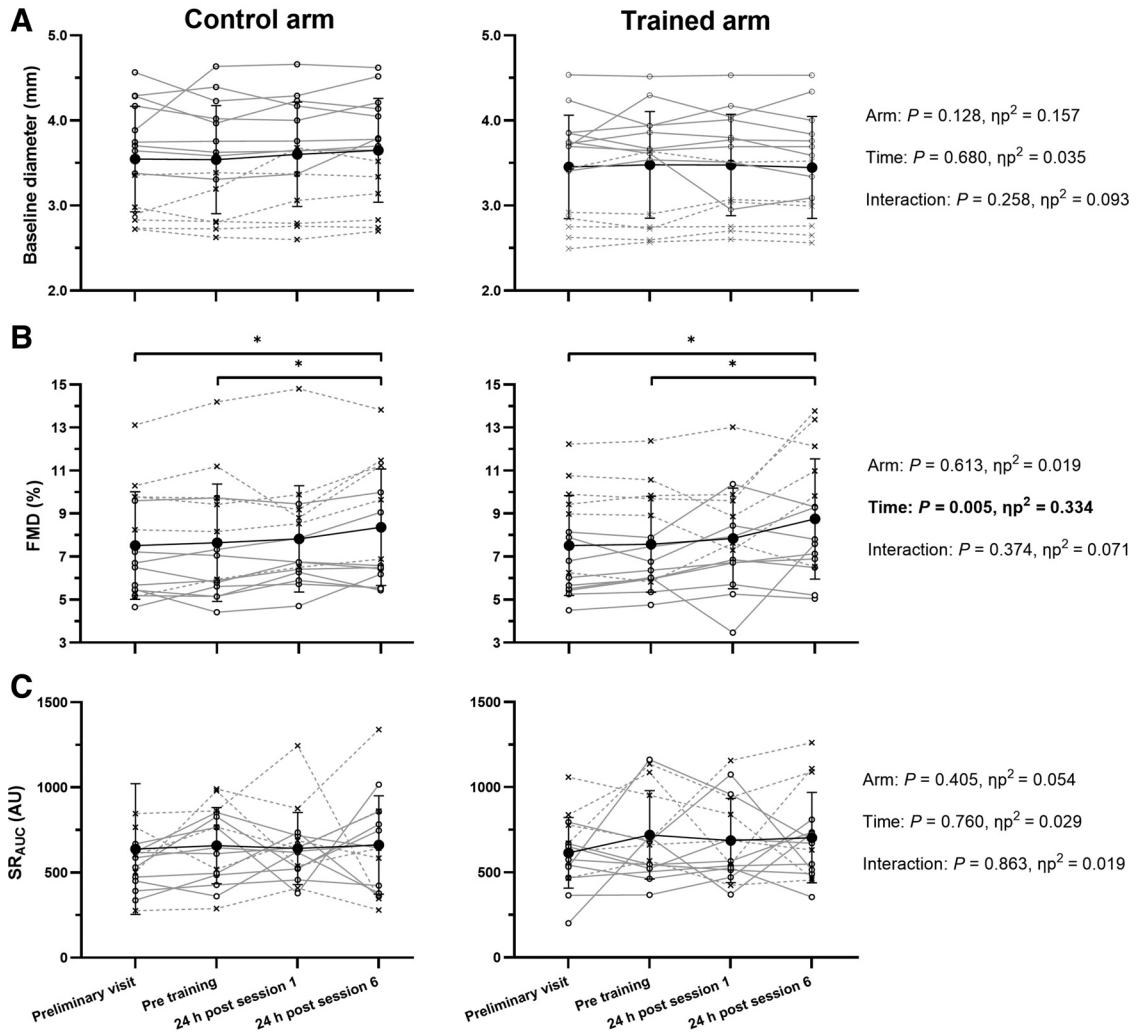

Figure 3. Macrovascular responses to 2 wk (6 sessions) of ischemic preconditioning (IPC) training. *A*–*C*: baseline diameter (*A*), flow- mediated dilation (FMD; *B*), and area under the curve for shear rate (SR_{AUC}; *C*). Data are presented for men ("o" and sold line) and women ("x" and dashed line) with means and SD in black.

to extrapolate our data beyond healthy young adults, but the observation that vascular function may be improved even in this group is encouraging.

Conclusions

We observed that the local and remote effects of IPC on vascular health were equivalent, and that the benefits to FMD may be greater with sustained IPC training, compared with a single exposure. Our data further support the use of IPC as a method to improve resting vascular function, even in healthy adults.

DATA AVAILABILITY

Raw data are available upon request.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

B.B., H.H., D.M.K., H.M., T.T., and A.T. conceived and designed research; B.B., H.H., D.M.K., A.B.L., H.M., T.T., and A.T. performed experiments; B.B., H.H., D.M.K., A.B.L., H.M., T.T., and A.T. analyzed data; B.B., H.H., D.M.K., A.B.L., H.M., T.T., and A.T. interpreted results of experiments; B.B. prepared figures; B.B. drafted manuscript; B.B., H.H., D.M.K., A.B.L., H.M., T.T., and A.T. edited and revised manuscript; B.B., H.H., D.M.K., A.B.L., H.M., T.T., and A.T. approved final version of manuscript.

REFERENCES

1. **Murry CE**, **Jennings RB**, **Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986. doi:10.1161/01.cir.74.5.1124.
2. **Przyklenk K**, **Bauer B**, **Ovize M**, **Kloner RA**, **Whittaker P.** Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 87: 893–899, 1993. doi:10.1161/01.cir.87.3.893.
3. **Loukogeorgakis SP**, **Panagiotidou AT**, **Broadhead MW**, **Donald A**, **Deanfield JE**, **MacAllister RJ.** Remote ischemic preconditioning provides early and late protection against endothelial ischemia-

reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol* 46: 450–456, 2005. doi:10.1016/j.jacc.2005.04.044.

4. **Moro L**, **Pedone C**, **Mondi A**, **Nunziata E**, **Antonelli Incalzi R.** Effect of local and remote ischemic preconditioning on endothelial function in young people and healthy or hypertensive elderly people. *Atherosclerosis* 219: 750–752, 2011. doi:10.1016/j.atherosclerosis.2011.08.046.
5. **Kim J**, **Franke WD**, **Lang JA.** Delayed window of improvements in skin microvascular function following a single bout of remote ischaemic preconditioning. *Exp Physiol* 106: 1380–1388, 2021. doi:10.1113/EP089438.
6. **Jones H**, **Hopkins N**, **Bailey TG**, **Green DJ**, **Cable NT**, **Thijssen DH.** Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans. *Am J Hypertens* 27: 918–925, 2014. doi:10.1093/ajh/hpu004.
7. **Jones H**, **Nyakayiru J**, **Bailey TG**, **Green DJ**, **Cable NT**, **Sprung VS**, **Hopkins ND**, **Thijssen DH.** Impact of eight weeks of repeated ischaemic preconditioning on brachial artery and cutaneous microcirculatory function in healthy males. *Eur J Prev Cardiol* 22: 1083–1087, 2015. doi:10.1177/2047487314547657.
8. **Thijssen DHJ**, **Bruno RM**, **van Mil ACCM**, **Holder SM**, **Faita F**, **Greyling A**, **Zock PL**, **Taddei S**, **Deanfield JE**, **Luscher T**, **Green DJ**, **Ghiadoni L.** Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 40: 2534–2547, 2019. doi:10.1093/eurheartj/ehz350.
9. **Atkinson G**, **Batterham AM**, **Thijssen DH**, **Green DJ.** A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens* 31: 287–291, 2013. doi:10.1097/HJH.0b013e32835b8164.
10. **Pyke KE**, **Tschakovsky ME.** Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *J Appl Physiol (1985)* 102: 1510–1519, 2007. doi:10.1152/japplphysiol.01024.2006.
11. **Bond B**, **Williams CA**, **Barker AR.** The reliability of a single protocol to determine endothelial, microvascular and autonomic functions in adolescents. *Clin Physiol Funct Imaging* 37: 703–709, 2017. doi:10.1111/cpf.12362.
12. **Wong BJ**, **Wilkins BW**, **Holowatz LA**, **Minson CT.** Nitric oxide synthase inhibition does not alter the reactive hyperemic response in the cutaneous circulation. *J Appl Physiol (1985)* 95: 504–510, 2003. doi:10.1152/japplphysiol.00254.2003.
13. **Doshi SN**, **Naka KK**, **Payne N**, **Jones CJ**, **Ashton M**, **Lewis MJ**, **Goodfellow J.** Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci (Lond)* 101: 629–635, 2001.
14. **Heusch G.** Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circ Res* 116: 674–699, 2015. doi:10.1161/CIRCRESAHA.116.305348.
15. **Depre C**, **Park JY**, **Shen YT**, **Zhao X**, **Qiu H**, **Yan L**, **Tian B**, **Vatner SF**, **Vatner DE.** Molecular mechanisms mediating preconditioning following chronic ischemia differ from those in classical second window. *Am J Physiol Heart Circ Physiol* 299: H752–H762, 2010. doi:10.1152/ajpheart.00147.2010.