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IMPACT OF 8 WEEKS OF REPEATED ISCHEMIC PRECONDITIONING ON BRACHIAL ARTERY AND CUTANEOUS MICROCIRCULATORY FUNCTION IN HEALTHY MALES

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ABSTRACT

BACKGROUND: Ischemic preconditioning (IPC) has well-established cardiac and vascular protective effects. Short interventions (1-week) of daily IPC episodes improve conduit and microcirculatory function. This study examined whether a longer (8-weeks) and less frequent (3 per-week) protocol of repeated IPC improves vascular function. **METHODS:** Eighteen males were randomly allocated to either IPC (22.4±2.3 yrs, 23.7±3.1 kg.m^2) or a control intervention (26.0±4.8 yrs, 26.4±1.9 kg.m^2). Brachial artery endothelial-dependent (FMD), forearm cutaneous microvascular function and cardiorespiratory fitness were assessed at 0, 2 and 8 weeks. **RESULTS:** A greater improvement in FMD was evident following IPC training compared to control at weeks 2 [2.24% (0.40, 4.08); *P*=0.02] and 8 [1.11% (0.13, 2.10); *P*=0.03]. Repeated IPC did not change cutaneous microcirculatory function or fitness. **CONCLUSIONs:** These data indicate that a feasible and practical protocol of regular IPC episodes improves endothelial function in healthy individuals within 2 weeks, and these effects persist following repeated IPC for 8 weeks.

INTRODUCTION

Ischemic preconditioning (IPC) is characterised by brief episodes of ischemia followed by reperfusion (single or multiple bouts) and offers cardiac and vascular protection against prolonged ischemia and ischemia reperfusion injury (IRI).[1, 2] Recent studies have examined daily exposure of IPC episodes as a therapeutic intervention and found enhanced brachial artery endothelial function[3], skin perfusion[3], resistance[4] artery function and protection from conduit artery induced IRI[5]. Therefore, daily IPC may potentially be a useful intervention to improve vascular function.

Cardiovascular protection from IPC is present for 1-2 h following an IPC episode (early phase). Whilst effects diminish after 24 h, they persist for 3-4 days (late phase)[6]. Given these characteristics, application of IPC on a daily basis may not be necessary. Furthermore, previous studies have examined relatively short periods of repeated IPC (≥1 week), which raises the question whether prolonged exposure to repeated IPC will maintain or further improve the effects on the vasculature. Therefore, we examined the impact of regular bouts of IPC (3 x per week) for 8-weeks on conduit artery and microcirculatory function in young healthy male individuals.

METHODS

Participants

Eighteen males, non-smokers, free from cardiovascular disease were recruited. The study was approved by the university ethics committee and conformed to the *Declaration of Helsinki*. All participants provided written informed consent.

Research Design

Participants were randomly assigned (computer-generated sequence) to IPC (*n*=9, 22.4±2.3 yrs, BMI 23.7±3.1 kg.m^2) or control (*n*=9, no intervention, 26.0±4.8 yrs, BMI 26.4±1.9 kg.m^2). The intervention-arm was counterbalanced and allocation to the intervention was performed prior to measurements. Assessment of, brachial artery function and cutaneous microcirculatory function on the arm assigned to the intervention was performed at week 0, 2 and 8. Participants fasted for 6 h, refrained from alcohol and exercise for 24 h and caffeine for 6 h before each visit.

Experimental Measurements

Following 30-min supine rest, images of the brachial artery using high-resolution ultrasound were acquired (Terason, t3000, Aloka, UK). Brachial artery endothelium-dependent vasodilation was assessed via flow mediated dilation (FMD). Diameter, flow, and shear stress were measured prior to, and following 5 min of forearm cuff inflation[7]. Analysis was performed using custom-designed edge-detection and wall-tracking software by single person following completion of the final participant who was blinded to the treatment allocation.[7, 8] Allometric scaling for baseline diameter was performed in our analysis. [9]

Red blood cell flux was measured on the forearm using laser-Doppler flowmetry (Periflux 5001, Sweden). Skin temperature was controlled with local heating units (Perimed 455, Sweden). Mean arterial pressure (MAP) was measured using finger photoplethysmography (Finapres Medical Systems, Netherlands). Following 10min of baseline, heating from 33 to 42ºC (0.5 ºC per 5 s) occurred and then held at 42ºC for 30 min. Cutaneous vascular conductance (CVC) was calculated as flux (mV) divided by MAP (mmHg).

A single observer measured height and body mass. Following a 2-min warm-up, the treadmill workload increased with step-wise increments in speed and gradient. VO_{2peak} was calculated from expired gas fractions (Oxycon Pro, Jaeger, Germany) as the highest

consecutive 15s period of oxygen uptake occurring in the minute before volitional exhaustion. Anthropometric and fitness measurements performed at week 0 and 8 only.

IPC Intervention: A cuff around the upper arm was inflated to 220 mmHg for 5 min using a rapid inflator to produce an ischemic stimulus, and then deflated to allow reperfusion for 5 min. This cycle was repeated 4 times per episode. Three episodes per week were administered for 8-weeks. Participants were supervised for each IPC episode.

Control Intervention: Normal routine, no alteration of physical activity or dietary habits.

Statistical analysis

Delta change from week 0 was calculated[10] and analysed using linear mixed modelling, with week 0 data as a covariate to account for any differences between groups using the Statistics Package for Social Sciences for Windows (SPSS Inc. Chicago, IL, USA). Data are presented as mean and 95% confidence intervals.

Results

Two individuals (1 IPC, 1 control) dropped out during the study. Individuals in the IPC-intervention (*n*=8) demonstrated 96% compliance. There were negligible differences in the change in MAP at week 2 [3 mmHg (-11, 15); *P*=0.72] and week 8 [1 mmHg (-10, 11); *P*=0.30] between IPC and control. Similarly, fitness [1.38 $ml.kg^{-1}.min^{-1}$ (-2.67, 5.43); *P*=0.47] and body mass index [0.37 $kg.m^2$ (-1.12, 0.37); *P*=0.98] did not change with the interventions.

Brachial artery FMD: A significant increase in FMD was evident following IPC at week 2 [2.24% (0.40, 4.08); *P*=0.02] and week 8 [1.11% (0.13, 2.10); *P*=0.03] compared to control. The intervention did not impact on brachial artery diameter, time-to-peak diameter and shear rate area-under-the-curve.

Cutaneous microcirculation: IPC did not significantly affect resting $CVC\%_{max}$ at week 2 [1.8% (-1.8, 5.4); *P*=0.30] or week 8 [0.41% (-2.0, 2.8), *P*=0.72] when compared to control. The local heating protocol induced the typical pattern of an initial peak, nadir and a plateau in skin flux during all assessments. IPC did not affect $CVC\%_{max}$ at the initial peak at week 2 [*P*=0.96] or week 8 [*P*=0.68], the nadir at week 2 [*P*=0.26] or week 8 [*P*=0.68] or plateau at week 2 [*P*=0.39] or week 8 [*P*=0.96].

Discussion

The novel finding of this study was that 8-weeks of IPC, enhanced brachial artery FMD, compared to a control group who received no intervention. Importantly, enhanced FMD was evident after only 2-weeks (i.e. 6 IPC sessions) and remained elevated after 8-weeks. Although we observed no effect on cutaneous perfusion, our data collectively suggest that a protocol of regular IPC is an effective and low-cost tool to improve conduit artery function in healthy individuals.

An important difference with previous reports is that we adopted only 3 IPC episodes per week compared to daily IPC.[3, 4] The design of less frequent repeated IPC, was informed by the timing of the late phase of protection, suggesting that daily episodes of IPC may not be necessary for optimal benefit.[6] Therefore by design, FMD improvements in our study coincide with the late phase of protection, which is believed to be induced by *de novo* synthesis of cardioprotective proteins, such as upregulation of the NO-pathway and cyclooxygenase-2.[11] However, mechanisms of cardioprotection of a single bout of IPC may substantially differ from those linked to the effects of repeated IPC.[12] Interestingly, application of exercise and/or heat exposure interventions of similar frequency and duration also improve brachial artery FMD in healthy individuals;[13-15] and may cause similar adaptations to improve vascular function. In support of this concept, one previous study has shown that an acute bout of exercise has preconditioning effects against IRI using an animal

model.[16] Preconditioning effects of an exercise bout has also been shown previously on cardiac function in patients with coronary artery disease.[17]

Early reports have raised concerns about the application of repeated IPC, suggesting repeated protocols may negate vascular protection.[18] Nevertheless, we found FMD was enhanced after 2-weeks (i.e. 6 IPC episodes) and remained significantly elevated after 8-weeks, indicating that the protocol of repeated IPC in healthy individuals for 8-weeks does not negate initial beneficial vascular adaptations. Whilst it is possible that the impact of IPC attenuates over time, this could be explained by structural changes in the vascular bed which occur after regular and repeated exposure to a stimulus [8].

We did not observe changes in cutaneous blood flow, which contrasts our previous findings with daily IPC[3] and reports of enhanced cutaneous blood flow following a single episode of IPC.[19] Our previous findings could be related reduced blood pressure,[3] which was not evident in the current study. Alternatively, the stimulus in the current study might not have been of sufficient frequency to induce adaption in the cutaneous circulation, such as that observed with daily intervention.

Improvements of vascular function is a central feature of various non-pharmacological interventions, importantly, increases in vascular function are associated with reductions in cardiovascular disease risk. A 1% increase in FMD is associated with a 13% reduction in cardiovascular events[20]. Our data therefore provide clinically meaningful insight, in that repeated exposure to episodes of IPC can improve FMD in young healthy individuals. These findings support future research to examine the impact of repeated IPC protocols in those with *a priori* vascular dysfunction (e.g. individuals with cardiovascular risk factors).

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