Title: Seven-day ischemic preconditioning improves muscle efficiency during cycling

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Abstract

Ischemic preconditioning (IPC) has emerged as a potential non-invasive ergogenic aid to enhance exercise performance. Repeated application of IPC has demonstrated clinical efficacy, therefore our aims were to investigate its effect on endurance cycling performance and muscle efficiency. Twenty participants undertook 7-d repeated bilateral lower limb occlusion (4 x 5-min) of IPC (220 mmHg) or sham (20 mmHg). Prior to and 72-h following the intervention, participants performed submaximal cycling at 70, 80 and 90 % of ventilatory threshold (VT) followed by an incremental exercise test. IPC had no effect on $\dot{V}O_{2max}$ (P = 0.110); however, time to exhaustion increased by ~ 9 % and W_{max} by ~ 5 % (IPC pre 307 ± 45 to post 323 ± 51 W) relative to sham (P = 0.002). There were no changes in gross efficiency (GE) (P > 0.05); however, delta efficiency (DE) increased by 3.1 % following IPC (P = 0.011). Deoxyhaemoglobin (HHb) was reduced following IPC ~ 30% (P = 0.017) with no change in total haemoglobin (tHb). Repeated IPC over 7-d enhanced muscle efficiency and extended cycling performance. The physiological effects of repeated IPC on skeletal muscle efficiency explains the notable improvements in endurance performance.

Keywords: NIRS, blood flow restriction, exercise, ischaemia, mitochondria, performance

Introduction

Ischemic preconditioning (IPC), involving intermittent cessation of blood flow followed by reperfusion, has emerged as a potential non-invasive ergogenic aid to enhance exercise performance (de Groot et al. 2010) and improve cardiovascular health (Thijssen et al. 2016). In tests of endurance (continuous exercise >100 s), the acute application of IPC has conferred beneficial effects, enhancing exercise capacity or performance in the range of 2 to 16 % (de Groot et al. 2010; Crisafulli et al. 2011; Bailey et al. 2012b; Kido et al. 2015; Cruz et al. 2015; Cocking et al. 2018). However, a number of studies have also reported no benefits of IPC on endurance performance (< 2 %) (Clevidence et al. 2012; Tocco et al. 2015; Sabino-Carvalho et al. 2017; Kilding et al. 2018). Whilst the mechanisms underlying the ergogenic properties of IPC are unclear inter-individual variability in the response to IPC has been reported, with a responder rate of ~67 % across 17 individual performance studies (Incognito et al. 2016), which may explain the inconsistencies reported.

Repeated application of IPC across a number of days, thereby increasing the stimulus, has demonstrated clinical efficacy in a dose-dependent manner (Yamaguchi et al. 2015). While a small number of studies have examined the repeated application of IPC on exercise performance (Foster et al. 2014; Lindsay et al. 2017, 2018), there has been no investigation of the associated physiological adaptations that might support an ergogenic effect. For example, notable improvements in systolic and diastolic blood pressure (Jones et al. 2014), vascular health (Jones et al. 2014) and skeletal muscle oxidative function (Jeffries et al. 2018), if translated to the exercising muscle, could enhance the efficiency of ATP turnover and increase blood flow, leading to improved tissue oxygenation and metabolite removal.

During repeated IPC, brief periods of ischemia increase oxidative stress (Yellon and Downey 2003), tissue hypoxia and muscle acidity (Sundberg and Kaijser 1992), whereas during reperfusion, the resultant hyperemic response increases sheer-stress on the vasculature (Tinken et al. 2010), which combine to initiate local muscle and vascular adaptations. IPC has been reported to protect against glycogen depletion (Lintz et al. 2013), reduce lactate production (Bailey et al. 2012b) and enhance oxidative function (Jeffries et al. 2018), whereby energy metabolism is favourably reduced during subsequent periods of ischemia (Addison et al. 2003). We have previously reported enhanced oxidative function following repeated IPC (Jeffries et al. 2018), leading us to speculate that adaptations in mitochondrial function may have occurred

(Jeffries et al. 2018). Indeed, IPC is effective in restoring mitochondrial dysfunction in skeletal muscle (Thaveau et al. 2007) and can modulate expression of electron transport chain proteins in myocardial tissue, thus improving mitochondrial efficiency (Cabrera et al. 2012). Although much of the mechanistic underpinnings remain to be explored during dynamic exercise, enhanced muscle oxidative properties are evident via accelerated muscle deoxygenation kinetics (Kido et al. 2015; Jeffries et al. 2018) and improved maintenance of muscle oxygenation (Patterson et al. 2015). While the potential for improved energy metabolism and muscle efficiency has recently been explored following acute application of IPC (Kilding et al. 2018), it is currently unknown whether repeated applications can elicit dose-dependent improvements in these critical endurance performance determinants.

The aim of this study was to investigate the effects of 7-d of repeated lower-limb IPC on endurance cycling performance, as well as evaluating changes in metabolic responses, efficiency and muscle oxygenation during exercise. Based on our above reasoning we hypothesized that relative to a sham control, 7-d repeated IPC would (i) improve cycling time to exhaustion, (ii) reduce oxygen extraction during submaximal exercise and, (iii) reduce the oxygen cost of submaximal exercise via enhanced muscle efficiency.

Material and Methods

Participants

Twenty healthy male participants volunteered to take part in this study (age 21 ± 2 years; height 181 ± 7 cm; body mass 84 ± 16 kg, $\dot{V}O_{2max}$ 45.0 ± 5.9 ml·kg^{-1}·min^{-1}) (Table 1). All participants were recreationally active and participated in university level team sports. They were non-smokers and not taking any medications. *A-priori* sample size was calculated using G*Power (Version 3.1.9.3). This was calculated according to changes in $\dot{V}O_{2max}$ 48-h post 7-d repeated IPC intervention [9.5 % increase; $P < 0.01$, $d = 0.91$, $n = 18$] (Lindsay et al. 2017). A minimum of eight participants per group was deemed a sufficient sample size in order to yield a power of 0.95 and $\alpha = 0.05$. A total of 20 participants were recruited to account for the possibility of dropout. Participants were asked to refrain from alcohol and caffeine consumption for 24-h and avoid strenuous exercise for 48-h prior to testing. Participants were told to maintain their current training regime for the duration of the study. All participants gave written informed consent. Ethical approval was provided by the institutional university ethics committee, which was conducted in accordance with the 1964 Helsinki declaration.

Experimental design

A randomized, single-blind, crossover design was adopted to examine the effect of IPC on cycling performance. Participants were assigned to an IPC or sham group using block randomization for groups of four participants at a time using online software (Research Randomizer (Version 4.0)) (Urbaniak and Plous 2015). Participants first visited the laboratory to provide informed consent, conduct baseline tests to determine the first ventilatory threshold (VT) and $\dot{V}O_{2max}$ (reported in table 1) and to familiarise with the IPC procedure used in the experimental trials. For the main experimental trial, participants reported to the laboratory on nine separate occasions, across 11 consecutive days. All trials were conducted at the same time of day to eliminate circadian variation.

Test of maximal aerobic capacity

All cycling exercise was performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands). The cycling setup was recorded on the first baseline visit and replicated for subsequent visits. During visit one (baseline testing), participants

undertook an incremental exercise test to volitional exhaustion to determine $\dot{V}O_{2max}$. Participants performed a 5-min warm-up at 80 W and selected their preferred cadence (85 ± 5 r·min^{-1}) which was then maintained for the duration of the study. The test started at 120 W and workload increased by 30 W·min^{-1} until volitional exhaustion. Expired gases were collected and measured using breath-by-breath expired air analysis (OxyCon Pro, Erich Jaeger GmbH, Hoechberg, Germany) with the highest average 30-s reported as $\dot{V}O_{2max}$. Achievement of $\dot{V}O_{2max}$ was considered as the attainment of at least two of the following criteria: (1) a plateau in $\dot{V}O_2$ despite increasing workload; (2) respiratory exchange ratio (RER) above 1.15; and (3) heart rate (HR) ± 10 beats·min^{-1} of predicted maximum HR calculated as 220-age (Howley et al. 1995). W_{max} was recorded as the highest power output averaged over 30-s recorded during the test and time to exhaustion was also recorded. Heart rate (HR) was recorded continuously throughout the trials (Polar Team System®, Polar UK). B[La] was measured 2-min post-test completion. The gas analyser was calibrated before every trial with gases of known concentration (15.95% O_2, 4.97% CO_2, BAL. N_2) and the turbine volume transducer was calibrated using a 3 L syringe (Hans Rudolph, Kansas City, USA). All subsequent $\dot{V}O_{2max}$ tests (visit 2 and visit 10) were performed in this way. Breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$ data from the incremental cycling test was used to plot ventilatory threshold (VT) using the simplified *v*-slope method (Schneider et al. 1993). From the determined VT, power outputs equating to 70 %, 80 % and 90 % of VT were calculated for subsequent exercise intensities to determine measures of cycling efficiency. Incremental ramp tests were performed at least one week prior to commencing the intervention protocol.

Determination of cycling efficiency

During visit 2 (pre-test) and visit 10 (post-test +72-h), participants exercised continuously at work rates of 70%, 80% and 90% of VT. Work rates were maintained for 5-min stages followed by 3-min rest periods (no pedalling). HR, B[La] and RPE were measured at the end of each 5-min period. $\dot{V}O_2$ and RER were averaged during the final 30-s period of each stage and related to work rate. Gross cycling efficiency (GE) was calculated as the ratio of work accomplished (W converted to kcal·min^{-1}) to energy expended (kcal·min^{-1}). Energy expenditure (EE) was calculated from $\dot{V}O_2$ and RER using the tables of Lusk (Lusk 1928). Delta efficiency (DE) was calculated as the reciprocal of the linear regression constant on an energy expended versus work rate plot (Coyle et al. 1992; Moseley and Jeukendrup 2001). Within-subject CV for

measures of absolute GE and DE are reported as 0.8 GE% and 1.7 DE%, respectively (Moseley and Jeukendrup 2001). After a 10-min rest period, an incremental ramp test was conducted, as previously described to examine $\dot{V}O_{2max}$, peak work rate and time to exhaustion (TTE).

NIRS device

A near-infrared spectroscopy (NIRS) optode (Portamon, Artinis Medical Systems, Zetten, the Netherlands) was placed on the vastus lateralis of the right leg (midway between the greater trochanter and the lateral epicondyle of the femur), shaved and secured with an elastic bandage (Tiger Tear, Hampshire, UK) to prevent movement and covered with an optically dense black material to minimize the intrusion of extraneous light. The vastus lateralis was chosen because it is one of the primary muscles active during the power phase of pedalling (Hug and Dorel 2009). The position of the probe was distal to the placement of the automatic inflation cuffs and was marked with indelible ink which was reapplied at regular intervals during the intervention protocol to ensure correct placement of the optode during the post intervention trial. During continuous cycling stages NIRS signals were obtained using a portable unit consisting of 3 channels (Portamon, Artinis Medical Systems, Zetten, the Netherlands). The system is a 2-wavelength continuous wave system that simultaneously uses the modified Beer-Lambert law and spatially resolved spectroscopy methods. Changes in tissue O_2Hb, HHb and tHb were measured using the differences in absorption characteristics of infrared light at 760 and 850 nm. Differential path factor (DPF) of 4 was used throughout. NIRS data was connected to a computer by Bluetooth for acquisition at 10 Hz. Values for HHb and tHb were reported as the delta from baseline (30-s average prior to cycling stage) to the final 30-s of each stage to examine the metabolic demands of the exercise bout. The HHb signal which is regarded as blood volume insensitive during exercise was therefore used to indicate intramuscular oxygenation status (De Blasi et al. 1994). Adipose tissue thickness (ATT) was measured at the site of the NIRS optode, in duplicate, to the nearest 0.1 mm using skinfold calipers (Harpenden, Burgess Hill, UK). The average value of skin and subcutaneous tissue thickness for the IPC group was 7.9 ± 2.6 mm and for the sham group 8.4 ± 2.5 mm (range of 4.8 - 12.9 mm). This was less than half the distance between source and the detector (35 mm).

IPC protocol

For the IPC protocol, automatic inflation cuffs (14.5 cm width – Delfi Medical Innovations, Vancover, Canada) were placed on the proximal portion of both thighs. The inflatable cuffs were connected to a pressure gauge and were automatically inflated to 220 mmHg (IPC) to ensure maximum occlusion across all participants (de Groot et al. 2010). The sham group experienced a lower pressure (20 mmHg) using the same automatic inflation cuffs. The protocol involved 5-min occlusion, followed by 5-min reperfusion, which was repeated four times (lasting 40-min) in the supine position. This procedure was repeated for seven consecutive days. To ensure the complete occlusion of arterial inflow to the limb, each individual had their limb occlusion pressure (LOP) assessed using a Doppler probe (UltraTec PD1, Ultrasound Technologies, Caldicot, UK) and automatic inflation cuff, previously described (Hughes et al. 2018). Average LOP is reported in Table 1.

Statistical analysis

Data are presented as means ± SD. To adjust for differences at baseline between groups we used an analysis of covariance (ANCOVA) (Vickers and Altman 2001) to determine the difference between GE, DE, $\dot{V}O_{2max}$, TTE, PO, HHb, tHb, HR, B[La] and RPE. There was one independent variable with two levels (IPC *vs*. sham), with the participants' pre-test baseline data used as a covariate. Magnitude of effects were calculated with partial eta-squared (η_p^2) according to the following criteria: 0.02, a small difference; 0.13, a moderate difference; 0.26 a large difference. Statistical analysis was performed using SPSS 21 (IBM, Armonk, NY). Data were presented with GraphPad Prism (GraphPad Software, La Jolla, CA). Statistical significance was set at $P < 0.05$.

RESULTS

Performance tests

$\dot{V}O_{2max}$ was not different following 7-d repeated IPC or sham (IPC pre 44.7 ± 5.1 to post 45.0 ± 5.6 ml·kg^{-1}·min^{-1}; sham pre 42.8 ± 7.3 to post 40.6 ± 8.1 ml·kg^{-1}·min^{-1}) ($F_{(1, 17)}$ = 2.838, P = 0.110, η_p^2 = 0.143). However, TTE was extended during the incremental ramp test by ~32-s (9%) (IPC pre 373 ± 89 to post 405 ± 103-s; sham pre 351 ± 67 to post 344 ± 69-s) ($F_{(1, 17)}$ = 16.116, P = 0.001, η_p^2 = 0.487). All participants in the IPC group increased their exercise capacity, with two demonstrating a change < 5-s, and the remainder ranging from (22 – 63-s) (Figure 1a). Participants achieved a ~ 5 % increase in W_{max} output (IPC pre 307 ± 45 to post 323 ± 51 W; sham pre 295 ± 31 to post 292 ± 35 W) ($F_{(1, 17)}$ =imitaiton 12.610, P = 0.002, η_p^2 = 0.426) (Figure 1b). Maximal HR was moderately increased (IPC pre 193 ± 13 to post 196 ± 10 b·min^{-1}; sham pre 193 ± 7 to post 187 ± 13 b·min^{-1}) ($F_{(1, 17)}$ = 5.697, P = 0.029, η_p^2 = 0.251), along with end B[La] (IPC pre 9.8 ± 3.4 to post 10.8 ± 1.8 mmol^{-1}; sham pre 9.0 ± 1.7 to post 9.5 ± 1.6 mmol^{-1}) ($F_{(1, 17)}$ = 19.761, P < 0.001, η_p^2 = 0.538).

Cycling efficiency

GE did not differ between conditions at each exercise intensity, 70% VT ($F_{(1, 17)}$ = 0.837, P = 0.373, η_p^2 = 0.047), 80% VT ($F_{(1, 17)}$ = 3.054, P = 0.099 η_p^2 = 0.152), 90% VT ($F_{(1, 17)}$ = 2.171, P = 0.159 η_p^2 = 0.132) (Table 2). However, the moderate effect sizes evident at higher exercise intensities (80 & 90% VT) suggest that there could be meaningful increases in the IPC group. Observed differences in pre-to-post GE following the IPC intervention were as follows: IPC pre-to-post difference: 70 % VT +0.05 %; 80 % VT +0.6 %; 90% VT +0.5%; relative to sham pre-to-post difference: 70 % VT +0.3 %; 80 % VT +0.2 %; 90% VT +0.2%) (Table 2). There were no differences in B[La] or RPE between conditions (P > 0.05) (Table 3). DE demonstrated a main effect between IPC and sham ($F_{(1, 17)}$ = 8.213, P = 0.011, η_p^2 = 0.326) (Figure 2). DE increased by 3.1 % from baseline in the IPC group relative to a 0.6 % change in the sham control (IPC pre 21.2 ± 0.0 % to post 24.3 ± 0.0 %; sham pre 23.6 ± 0.1 % to post 24.2 ± 0.1 %).

Muscle oxygenation

IPC reduced delta HHb at exercise intensities of 70% VT ($F_{(1, 16)}$ = 9.610, P = 0.007, η_p^2 = 0.375), 80% VT ($F_{(1, 16)}$ = 8.634, P = 0.010, η_p^2 = 0.350) and 90% VT ($F_{(1, 16)}$ = 7.038, P = 0.017, η_p^2 = 0.305) compared to sham (Table 4). In contrast, tHb was not different between condition at 70% VT ($F_{(1, 16)}$ = 0.280, P = 0.604, η_p^2 = 0.017), 80% VT ($F_{(1, 16)}$ = 0.169, P = 0.686, η_p^2 = 0.010) and 90% VT ($F_{(1, 16)}$ = 0.379, P = 0.547, η_p^2 = 0.023). A representative figure illustrates no apparent change in tHb (Figure 3a) and a reduction in HHb during each cycling intensity for one participant (Figure 3b).

Discussion

The principle findings of this investigation were that following 7-d repeated IPC i) maximal exercise performance was improved by +9 %, without any change in $\dot{V}O_{2max}$, ii) DE was enhanced by +3.1 % and iii) there was an increase in tissue oxygenation during submaximal cycling tasks, with respect to sham. Together, these novel findings indicate that the physiological adaptations that occur following repeated IPC on skeletal muscle efficiency translate to improvements in endurance performance.

Acute application of IPC is reported to have inconsistent effects in tests of aerobic capacity. For example, some have reported no change in $\dot{V}O_{2max,}$ despite performance improvements (Crisafulli et al. 2011; Bailey et al. 2012a; Kilding et al. 2018), whilst others have reported a ~3 % increase in $\dot{V}O_{2max}$, alongside improvements in performance (de Groot et al. 2010; Cruz et al. 2015). Here, we can confirm that repetitive application of IPC across seven days did not confer any further changes to $\dot{V}O_{2max}$. However, repeated IPC did enhance performance power (W_{max}) by +9 %. These observations were made beyond the late phase (72-h) of IPC-mediated protection and, therefore, importantly do not reflect the acute effects of IPC (Yellon and Baxter 1995; Loukogeorgakis et al. 2005). Whilst there is limited research examining the repeated nature of IPC on performance, our findings contrast the +13 % increase in $\dot{V}O_{2max}$ reported in untrained individuals ($\dot{V}O_{2max}$ ~ 39 ml·kg^{-1}·min^{-1}) (Lindsay et al. 2017), but are further supported by work from the same group where no change in $\dot{V}O_{2max}$ was observed in moderately fitter individuals ($\dot{V}O_{2max}$ ~ 50 ml·kg^{-1}·min^{-1}), both immediately and 7-d following a repeated IPC procedure (Lindsay et al. 2018). Caution should be exercised when interpreting the large effects reported, to date, due to potential training effect in the recreational population, which was noted by the authors (Lindsay et al. 2017). The changes in performance power described are typically explained by increased $\dot{V}O_{2max,}$ improvements in lactate threshold and/or efficiency (Joyner and Coyle 2008). However, an inverse relationship has been reported between training-induced changes in exercise efficiency and $\dot{V}O_{2max}$ (Hopker et al. 2012). Thus, assuming the generalizability of this phenomenon, improved efficiency could explain the +9 % increase in $W_{max,}$ in the absence of a change in $\dot{V}O_{2max}$.

Efficiency is a measure of work generated, expressed as a percentage of total metabolic energy expended (Ettema and Loras 2009) and can provide insight into the metabolic processes

involved in work production. Following 7-d repeated IPC, GE appeared to increase as a function of intensity by +0.05 %, +0.6 % and +0.5 % at 70, 80 and 90 % VT, respectively (Table 2). Whilst these changes were not significant the higher exercise intensities showed moderate effect sizes and the increases reported are comparable to increases (+0.8 %) in GE in response to altitude training protocols (23-d live high: train low) (Gore et al. 2001) although less than those that can be achieved following a 6-week period of high-intensity training (+1.6 %) (Hopker et al. 2010). GE is known to increase with exercise intensity, representing a transition from less-to-more efficient states (Sidossis et al. 1992; Chavarren and Calbet 1999). Therefore, optimising mechanical power at higher work intensities may have enabled the effects of the IPC intervention to manifest. The small differences described could be related to the calculation of GE, which incorporates basal energy costs, the cost of respiratory and stabilizing muscles, as well as the internal mechanical work associated with exercise (Moseley and Jeukendrup 2001). Indeed there would be a reduced contribution of resting $\dot{V}O_2$ to whole-body $\dot{V}O_2$ as muscle contractions increase (Chavarren and Calbet 1999) and, therefore, GE is limited by the assumption that these metabolic processes are stable across a range of exercise intensities (Ettema and Loras 2009). However, such changes could also be explained by prior exercise at 70 % VT, which could have altered the interaction between oxygen delivery ($\dot{Q}O_2$) and utilization ($m\dot{V}O_2$), thus enhancing efficiency in subsequent bouts (Ferreira et al. 2005). It is also important to note that the within-subject CV for measures of GE has been reported as 0.8 GE% (Moseley and Jeukendrup 2001) and, therefore, could also be accounted for by measurement error.

The determination of muscle efficiency is better explained by DE (Coyle et al. 1992). DE examines the increase in external power and metabolic rate with increasing work rate and is less likely to be affected by changes in baseline metabolic processes (Ettema and Loras 2009). Therefore, DE may better reflect adaptations to local skeletal muscle, proximal to site where IPC was applied. The most striking finding of the present study was a +3.1 % increase in DE following 7-d repeated IPC. Indeed, these improvements in muscle efficiency confirm our previous work, where resting muscle metabolism was reduced by -16 % following 7-d repeated IPC (Jeffries et al. 2018). Whilst smaller changes in DE have been reported following 3-d nitrate supplementation (0.8 %) (Larsen et al. 2007), strength training in young cyclists (0.9 %) (Louis et al. 2012) and 6 weeks high-intensity training (~1 %) (Hopker et al. 2010), efficiency is known to differ according to training status and age (Hopker et al. 2013). The

participants recruited in this study were young (21 ± 2 y); however, they were untrained ($\dot{V}O_{2max}$ 45.0 ± 5.9 ml·kg^{-1}·min^{-1}) and hence may have a greater capacity for improvement. Among older athletes, where baseline measures of DE are reduced (~ 2 %) compared to younger athletes, a 3-week strength training programme achieved a +3.2 % increase in DE (Louis et al. 2012). Thus, the changes reported herein are feasible and most likely reflect the training status of the participants recruited. Our findings contrast those reported after acute application of IPC, where no changes in running economy (Kaur et al. 2017) or cycling economy (Clevidence et al. 2012; Kilding et al. 2018) have been reported. Furthermore, increasing the number of ischemic episodes during an acute application (up to 8 x 5-min), beyond the traditional 4 x 5-min protocol, elicited no additional improvements in performance (Cocking et al. 2018). Therefore, we suggest that repeated application of the IPC stimulus is imperative to elicit the longer term adaptations in the skeletal muscle observed in the current study and previously (Jeffries et al. 2018), which contribute to improved endurance capacity. Indeed, the benefits of repeated IPC are supported by work in animal models that suggest greater protection can be achieved against ischemia in a dose-dependent manner (Yamaguchi et al. 2015). However, more research is needed in this area to examine the dose-response effect of IPC and the minimal stimulus required to achieve these positive adaptations to facilitate exercise performance.

Near-infrared spectroscopy enabled further interrogation of $\dot{Q}O_2$ and $m\dot{V}O_2$ during the submaximal exercise protocols used in this study. The HHb signal, which is regarded as blood volume insensitive, is used to report muscle fractional oxygen extraction during exercise (De Blasi et al. 1993). Here, across a range of submaximal intensities, we observed a ~ 30 % reduction in the HHb signal following 7-d repeated IPC. This reduction suggests an increased fraction of oxygen in the muscle, which may represent a reduction in $m\dot{V}O_2$, $\dot{Q}O_2$, or a combination of these factors. However, tHb which represents the total hemoglobin concentration in the tissue (i.e. blood volume), did not significantly increase at higher work intensities (Table 4). We have previously established enhanced oxidative capacity and vascular improvements in the skeletal muscle following 7-d repeated IPC (Jeffries et al. 2018). Thus, enhanced muscular efficiency could result from an improved match between $m\dot{V}O_2$ and $\dot{Q}O_2$ in the skeletal muscle. IPC could therefore optimise the $\dot{Q}O_2/m\dot{V}O_2$ ratio to improve muscular efficiency at sub maximal intensities.

There is considerable heterogeneity in the correlation between $\dot{Q}O_2$ and $m\dot{V}O_2$, particularly in untrained individuals (Kalliokoski et al. 2005), suggesting a potential for mismatch between oxygen delivery and demand. Indeed, the balance of $\dot{Q}O_2/m\dot{V}O_2$ is modulated according to the metabolic requirements associated with increases in exercise intensity (Okushima et al. 2016). By examining the constituents of the Fick equation ($m\dot{V}O_2 = \dot{Q}O_2$ x [(a-v)O_2]), a reduction in arteriovenous O_2 difference [(a-v)O_2] or fractional oxygen extraction as indicated by NIRS, could also suggest that adaptations occurred in the muscle itself. Two possible mechanisms could explain these changes; a reduction in metabolic rate (reduced ATP requirement) or increased mitochondrial efficiency (increased ATP per molecule O_2). Indeed, preconditioned tissues demonstrate a slower rate of ATP depletion, milder acidosis, and, via the release and delivery of vasoactive triggers (VEGF, adenosine, bradykinin, NO), augmented peripheral vascular blood flow (Yellon and Downey 2003; Jones et al. 2014) and angiogenesis (Yellon and Downey 2003; Thijssen et al. 2016).

Limitations

There was clear inter-individual variability in the response to IPC during the $\dot{V}O_{2max}$ test in the current study. Three participants demonstrated negligible improvements in performance time < 8 s and W_{max} < 8 W and four individuals reported changes > 50 s and > 26 W, with mean changes being ~ 31 s and ~ 16 W respectively (Figure 1). These data support a ~ 70 % response rate, which aligns with a recent systematic review that reported the IPC responder rate at ~67% (Incognito et al. 2016). It should be noted that high responders may have influenced the general trends we report. In addition, baseline differences in body mass, $\dot{V}O_{2max}$ and W_{max} were also observed suggesting that collectively the sham group may have had a reduced training status. This group showed a small systematic reduction in performance time and W_{max} following the sham condition, but is unclear why. Although we statistically controlled for differences at baseline, it is possible that fitness status could have influenced the ability to respond to the IPC stimulus. A greater understanding is required to explore the physiological and psychological factors that may predispose an individual to the beneficial effects of IPC, both from a performance and clinical perspective.

It should be noted that some concerns exist regarding excessive bouts of IPC, termed ‘hyperconditioning’, which have been suggested to confer adverse effects at higher doses and

would be particularly relevant in susceptible populations (Whittaker and Przyklenk 2014). However, when administered as a series of brief occlusions, no side-effects have been reported for a single application, nor for repeated applications up to 300 days (Meng et al., 2012). IPC also remains an effective technique to elicit cardio-protection in patients undergoing cardiac surgery (Hausenloy and Yellon 2011).

Practical applications

One of the most interesting aspects of the current study is the change in efficiency, without any additional training load placed on the participants. IPC is also a simple, non-invasive technique that may confer competitive advantage. Furthermore, these changes occur in a short time frame (7 days) and, thus, could be utilized by athletes during preparation for competition. Whilst the magnitude of improvement may appear small, it could markedly influence performance in prolonged cycling. Future studies should consider the underlying mechanisms that support these improvements in muscle efficiency and the optimal dose required to elicit performance enhancement.

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Table 1: Participant characteristics. Data are presented as mean ± SD.

Participant characteristics	**IPC (*n* = 10)**	**Sham (*n* = 10)**
Age (y)	22 ± 3	21 ± 2
Height (cm)	180.4 ± 7.1	181.3 ± 6.3
Body mass (kg)	80.7 ± 10.3	87.5 ± 20.6
Baseline $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	46.0 ± 3.8	43.8 ± 7.6
Baseline peak power (W)	377 ± 89	296 ± 29
LOP (mmHg)	201 ± 13	188 ± 10

Participant characteristics are reported from baseline tests. Abbreviations: IPC, ischemic preconditioning, LOP, limb occlusion pressure.

Table 2. Gross efficiency (GE) during cycling at relative work rates (n = 20).

	GE (%)		
	IPC Pre	IPC Post	Δ
70% VT	19.6 ± 1.9	19.6 ± 1.5	0.05
80% VT	19.4 ± 1.7	20.0 ± 1.6	0.6
90% VT	19.8 ± 1.6	20.3 ± 1.5	0.5
	Sham Pre	Sham Post	
70% VT	17.6 ± 1.0	17.9 ± 0.9	0.3
80% VT	17.8 ± 0.8	18.0 ± 0.8	0.2
90% VT	18.4 ± 0.9	18.6 ± 0.9	0.2

Values are presented as mean ± SD. Δ represents the delta between pre and post measures.

Table 3. Blood lactate concentration (B[La]) and rating of perceived exertion (RPE) during cycling at relative work rates (n = 20).

	B[La]		RPE	
	IPC Pre	IPC Post	IPC Pre	IPC Post
70% VT	4.8 ± 1.7	4.9 ± 1.6	12.0 ± 1.7	12.4 ± 1.3
80% VT	6.5 ± 2.3	6.7 ± 2.3	15.0 ± 1.7	14.8 ± 1.6
90% VT	7.9 ± 2.9	8.2 ± 2.4	17.4 ± 1.5	17.4 ± 1.6
	Sham Pre	Sham Post	Sham Pre	Sham Post
70% VT	4.6 ± 1.3	4.6 ± 1.3	12.0 ± 2.3	11.4 ± 2.2
80% VT	6.3 ± 1.8	5.9 ± 1.4	14.3 ± 1.2	14.6 ± 1.6
90% VT	8.1 ± 2.1	7.8 ± 1.7	16.5 ± 1.5	16.7 ± 1.3

Values are presented as mean ± SD.

Table 4. Delta changes in deoxyhaemoglobin (HHb) and total haemoglobin (tHb) during cycling at submaximal exercise intensities. IPC (n = 9) and sham (n = 10).

	[HHb] delta (a.u.)			[tHb] delta (a.u)		
	IPC Pre	IPC Post	Δ	IPC Pre	IPC Post	Δ
70% VT	7.17 ± 5.12	4.87 ± 4.55 *	-2.3	-1.73 ± 8.27	-1.39 ± 5.85	-0.3
80% VT	7.78 ± 4.86	4.88 ± 3.76 *	-2.9	0.95 ± 7.81	1.03 ± 5.30	0.1
90% VT	7.76 ± 4.76	5.91 ± 3.96 *	-1.9	0.33 ± 7.87	1.20 ± 4.38	0.9
	Sham Pre	Sham Post		Sham Pre	Sham Post	
70% VT	7.93 ± 4.27	7.67 ± 3.75	-0.26	-0.52 ± 6.34	-0.31 ± 5.90	-0.2
80% VT	8.89 ± 4.81	8.24 ± 3.85	-0.65	2.26 ± 6.62	2.56 ± 6.09	0.3
90% VT	10.05 ± 4.93	9.87 ± 5.34	-0.18	3.36 ± 7.46	2.76 ± 7.11	0.6

Values are presented as mean ± SD. HHb, deoxygenated haemoglobin; tHb, total haemoglobin; a.u., auxillary units; VT ventilatory threshold. Δ represents the delta between pre and post measures * $P < 0.05$ post intervention differences from baseline.

Figure legends

Figure 1. The change in a) time to exhaustion (TTE) and b) peak power output (W_{max}) during an incremental ramp test following 7-d repeated IPC or sham (n = 20). * $P < 0.05$.

Figure 2. Percent change in pre-to-post delta efficiency (DE) following 7d IPC or sham. ** $P = 0.011$.

Figure 3. Representative exercise protocol showing cycling stages at 70% VT, 80% VT and 90% VT. Data shown is from NIRS HHb and presented as pre (BLACK) and post (GREY) 7-day repeated IPC.

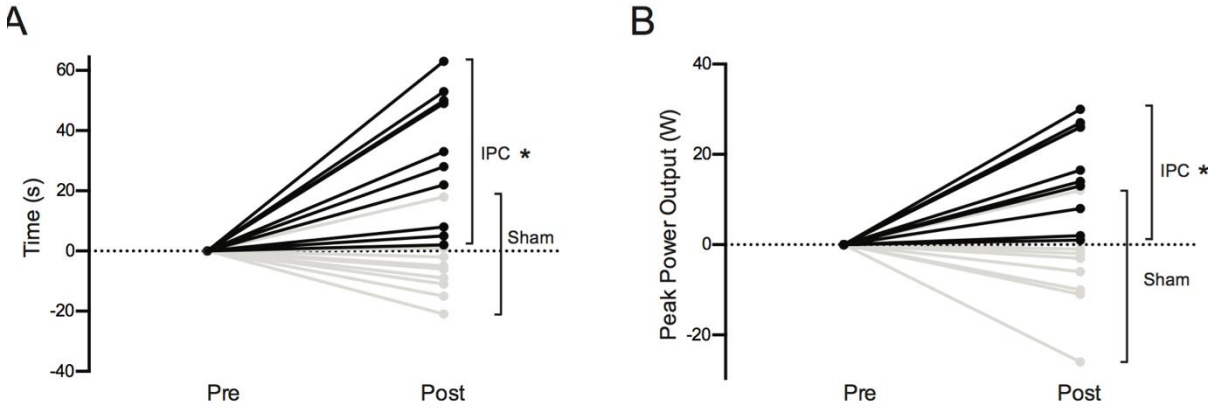
A
60
40
20
0
-20
-40
Time (s)
IPC *
Sham
Pre
Post
B
40
20
0
-20
Peak Power Output (W)
IPC *
Sham
Pre
Post

Figure 1

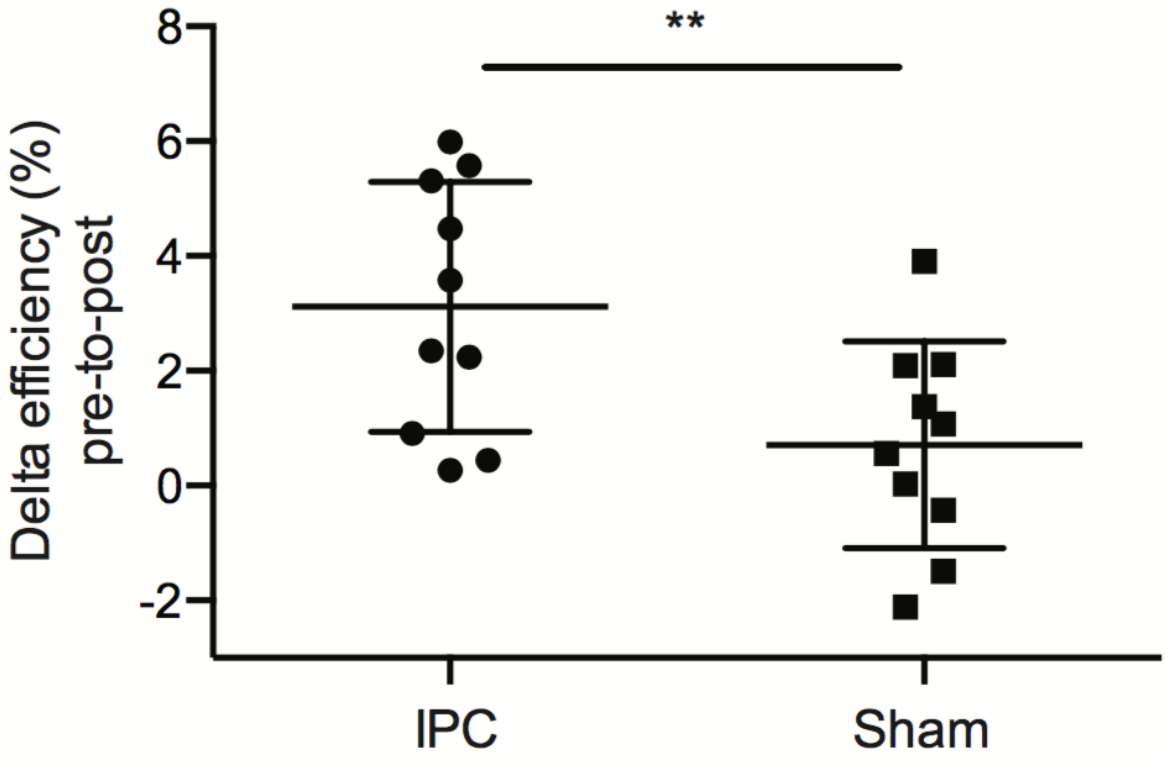

Figure 2

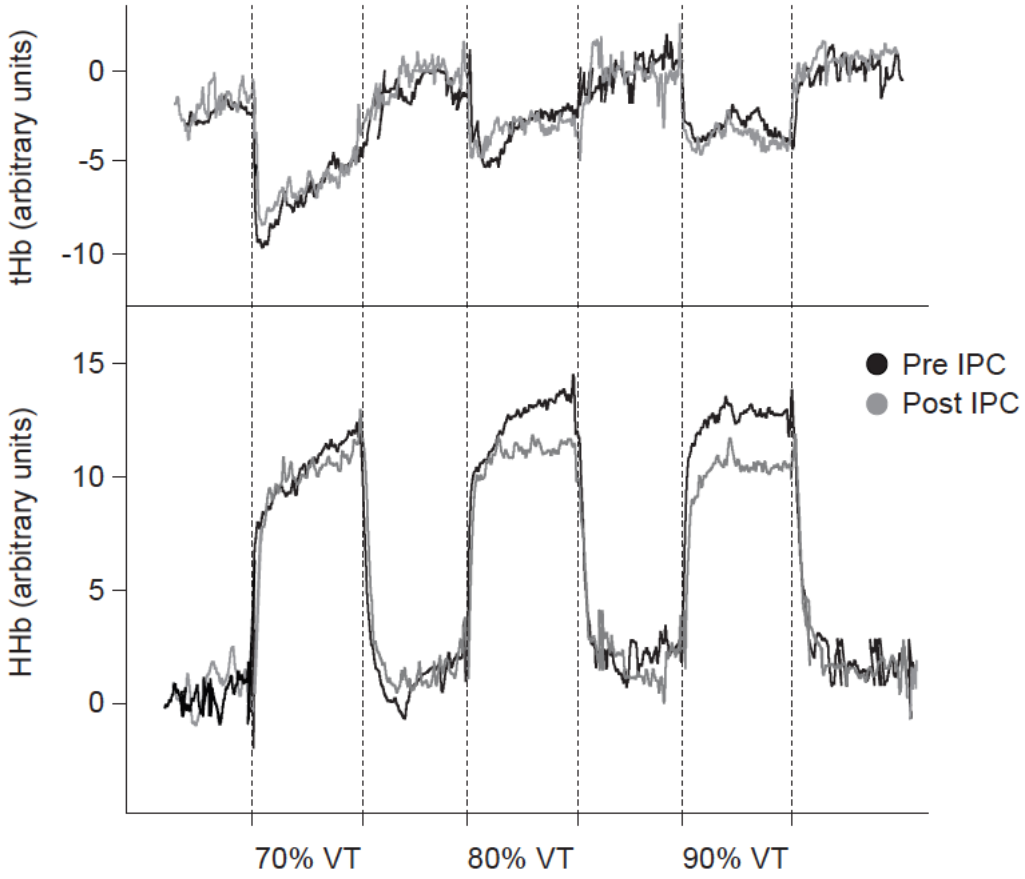
tHb (arbitrary units)
0
-5
-10
HHb (arbitrary units)
15
10
5
0
Pre IPC
Post IPC
70% VT
80% VT
90% VT

Figure 3