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Repeated Ischemic Preconditioning Effects on Physiological Responses to Hypoxic Exercise

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## Abstract

**Introduction** Repeated ischemic preconditioning (IPC) can improve muscle and pulmonary oxygen on-kinetics, blood flow and exercise efficiency but these effects have not been investigated severe hypoxia. The aim of the current study was to evaluate the effects of 7 d of IPC on resting and exercising muscle and cardio-pulmonary responses to severe hypoxia.

**Methods** Fourteen subjects received either: 1) 7 d of repeated lower-limb occlusion (4 x 5 min,  $217 \pm 30$  mm Hg) at limb occlusive pressure (IPC) or SHAM (4 x 5 min, 20 mm Hg).

Subjects were tested for resting limb blood flow ( $\dot{V}O_2$ ), relative microvascular deoxyhemoglobin concentration ([HHB]) and pulmonary oxygen ( $\dot{V}O_{2p}$ ) responses to steady state and incremental exercise to exhaustion in hypoxia (fractional inspired  $O_2 = 0.103$ ), which was followed by 7 d of IPC or SHAM, and retesting 72 h post intervention. **Results**

There were no effects of IPC on maximal oxygen consumption, time to exhaustion during the incremental test or minute ventilation and arterial oxygen saturation. However, the IPC group had higher delta efficiency based on pooled results and lower steady state delta[HHB] (IPC ~24% vs. SHAM ~6% pre-to-post), as well as slowing the [HHB] time constant (IPC ~26% vs. SHAM ~3% pre-to-post) and reducing the overshoot in [HHB]: $\dot{V}O_2$  ratio during exercise onset. **Conclusions** Collectively, these results demonstrate that muscle  $O_2$  efficiency and microvascular  $O_2$  distribution can be improved by repeated IPC but there are no effects on maximal exercise capacity in a severe hypoxia.

**Keywords:** conditioning; oxygen kinetics; near-infrared spectroscopy; hypoxic

## Introduction

Reductions in atmospheric pressure, as encountered at high-altitude, or simulated fractions of inspired oxygen ( $F_iO_2$ ) lead to corresponding reductions in the partial pressure of oxygen in the atmospheric and, thus, inspired air.<sup>12</sup> These changes perturb the  $O_2$  transport cascade, which can often reduce arterial oxygen content,<sup>4</sup> impairing exercise capacity and maximal oxygen consumption ( $\dot{V}O_{2max}$ ).<sup>3, 42</sup> However, the reported reductions in arterial oxygen content in response to severe hypoxia cannot fully explain the observed reduction in  $\dot{V}O_{2max}$ . Thus, reduced cardiac output ( $\dot{Q}$ ) and skeletal muscle blood flow are the most commonly ascribed factors that contribute to reduced  $\dot{V}O_{2max}$ .<sup>2</sup> Furthermore, the reduced pulmonary diffusion gradient also acutely disrupts the on-transient pulmonary  $\dot{V}O_2$  and muscle microvascular response to moderate-intensity exercise, such that mismatches in  $O_2$  delivery and utilization have been reported.<sup>39, 46</sup> The  $O_2$  delivery– $\dot{V}O_2$  matching is vital in setting blood–myocyte  $PO_2$ , thus directly influencing oxidative metabolic regulation.<sup>23</sup> These factors limit exercise capacity at altitude but could feasibly be attenuated by targeted training interventions.

Given that many occupations (i.e. military, emergency services and sport) might require their personnel to perform physical tasks in hypoxic conditions, such as that experienced at high-altitude, it is important that the efficacy of practical, time and cost effective strategies are investigated to help prepare for exercise in hypoxic environments. For example, ischemic preconditioning (IPC) can enhance endurance exercise performance in normoxia<sup>8, 19, 22</sup> and hypoxia ( $F_iO_2 \sim 0.12-0.16$ ).<sup>7, 10, 33</sup> This includes  $\sim 13\%$  increases in peak  $\dot{V}O_2$  following 1 wk of repeated IPC.<sup>24</sup> While acute pre exercise IPC interventions have been reported to enhance lower limb oxygenation in hypoxia<sup>7</sup> and might partly support the ergogenic effect,

contradictory evidence has been presented.<sup>11,16,43</sup> These inconsistent findings could relate to the mode of IPC, with repeated IPC (consecutive daily interventions) eliciting the greatest effect on limb blood flow, muscle oxidative capacity and subsequent normoxic endurance performance.<sup>19,20,21</sup> The most apparent physiological and ergogenic effects on running performance (~ 7 %) have been reported following repeated IPC (5 d) at higher outdoor altitudes (3560 m - 4342 m; ~ F<sub>I</sub>O<sub>2</sub> ~ 0.13–0.12, respectively);<sup>10</sup> however, the study was unavoidably limited in its exercising measurements, owing to the outdoor testing environment and the chronic IPC adaptation window was not strictly investigated.

The lack of attention towards repeated IPC and hypoxic exercise tolerance is surprising, given the common physical challenges that take place under these conditions<sup>28,38</sup> and the dose dependent evidence presented in both clinical<sup>21,44</sup> and performance domains.<sup>19,20,24</sup> The adaptations conferred by IPC would be advantageous to offset the deleterious effects of hypoxia on oxidative capacity and efficiency<sup>17</sup> and could preferentially adjust the transient muscle microvascular O<sub>2</sub> distribution limitation observed in hypoxic exercise.<sup>39</sup> The most recent findings demonstrate that 7 d of remote IPC enhances delta efficiency and muscle microvascular O<sub>2</sub> extraction, leading to improved exercise tolerance in normoxia. Indeed, our research group has also demonstrated increased DE following 5-days IPC combined with heat acclimation<sup>41</sup>, which is an indication of muscle efficiency.<sup>6</sup> DE examines the global relationship between metabolic rate and external power across increasing intensities, and is less likely to be affected by changes in baseline metabolic processes<sup>9</sup>. Furthermore, based on the tightly coupled relationship reported between phase II pulmonary  $\dot{V}O_2$  kinetics (the time constant of  $\dot{V}O_2$  from rest to exercise) and the matching of O<sub>2</sub> delivery– $\dot{V}O_2$ <sup>39</sup> and the capacity to enhance these parameters with physical training<sup>31</sup>, it would be logical that collective improvements in these parameters would occur alongside faster pulmonary on-

kinetics. This could also enhance exercise tolerance via reductions in the slow component, which should theoretically grow in importance as the exercise duration or intensity increases.

<sup>30</sup> Therefore, the aim of the current study was to evaluate the effects of repeated daily IPC (7 d) conducted in normoxic environments, on resting and exercising muscle and cardio-pulmonary responses to severe hypoxia ( $F_{iO_2} \sim 0.103$ ). We hypothesized that repeated IPC would enhance the muscle microvascular and pulmonary  $\dot{V}O_2$  kinetic response and exercise efficiency, leading to increased time to exhaustion during incremental exercise.

## **Methods**

### ***Subjects***

Fourteen recreationally-trained males, who were familiar with laboratory based exercise (age,  $26 \pm 4$  y; stature,  $181 \pm 7$  cm; body mass,  $83.1 \pm 12.0$  kg; BMI  $24.9 \pm 1.6$ ) were recruited. Table I demonstrates baseline subject characteristics by group and *t*-tests showed no differences between groups. A sample size of 6 per group was calculated using G\*Power (Version 3.1.9.3), according to reductions in exercising delta deoxyhemoglobin concentration ( $\Delta[Hb]$ ) post 7 d repeated IPC intervention ( $d = 1.64$ ; '*Large*').<sup>19</sup> Accordingly, 7 subjects were recruited in each group to account for potential attrition, although no subjects dropped out of the study. Subjects were non-smokers and had not spent any time at altitude  $> 1000$  m in the past 6 months. Institutional ethical approval was provided by St Mary's University ethics committee, which was conducted in accordance with the 2013 Helsinki declaration.

**\*\*\*Insert Table I here\*\*\***

## *Design*

Subjects were block randomized into 1 of 2 equal groups: either 7 consecutive days of IPC or SHAM intervention. As demonstrated in Figure 1, subjects visited the laboratory for baseline testing, followed by 7 consecutive intervention visits and 1 post testing visit, which occurred 72 h after the final intervention (9 visits over 15 d). The 72-h time period post-intervention was used as this is outside the reported early and late phases of protection conferred by IPC<sup>26</sup> and, therefore, would reflect a sustained adaptation to the IPC stimulus. The pre and post tests comprised a brief pre exercise assessment of limb blood flow, followed by steady state cycling (mechanical power output of 100 W) at moderate exercise intensities in a hypoxic environmental chamber, simulating high altitude ( $F_{iO_2} = 0.103$ ; simulated altitude ~ 5750 m), which was temperature and humidity controlled (18 °C, 40% Relative Humidity [RH]). This was followed by an immediate transition into an incremental ramp test to exhaustion. This  $F_{iO_2}$  was chosen as it ensured that simulated conditions were a similar severity to the most comparable repeated IPC study (0.12-0.13  $F_{iO_2}$ ),<sup>10</sup> and lower than the level shown to impair muscle and pulmonary kinetics (0.15  $F_{iO_2}$ ).<sup>39, 46</sup>

**\*\*\*Insert fig 1 here\*\*\***

## *Procedure*

A NIRS optode (Portamon, Artinis Medical Systems, Zetten, The Netherlands) was fitted to the medial gastrocnemius of the right leg prior to entering the chamber during the baseline and post testing visits and remained on for the duration of these visits. Specifically, the NIRS device was fitted 2/3 distance from the calcaneus and anterior fossa and secured with an elastic bandage (Tiger Tear, Hampshire, United Kingdom) to prevent movement and covered

with an optically dense black material to minimize the intrusion of extraneous light. The position of the probe 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. Changes in tissue oxyhemoglobin ( $[O_2Hb]$ ),  $[HHb]$  and total hemoglobin ( $[tHb]$ ) relative concentrations were measured ( $\mu M$ ) using a spatially resolved spectrometer, measuring the differences in absorption characteristics of infrared light at 760 and 850 nm, with data acquisition of 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 the steady state trial ( $\Delta[HHb]_{ss}$  and  $\Delta[tHb]_{ss}$ , respectively) and between the end of the steady state trial to the end of the incremental ramp test ( $\Delta[HHb]_{inc}$  and  $\Delta[tHb]_{inc}$ , respectively) to examine the local muscle metabolic demands of the exercise bout.

Resting limb blood flow (LBF) was measured to identify muscle microvascular blood flow in response to the severe hypoxic environment, immediately prior to the rest-to-exercise transition. This was intended to evaluate the rested state of the muscle microvasculature when exposed to a severe hypoxic stimulus, in the period closest to the exercise transition, which we hypothesized could have been affected by the IPC intervention. Subjects entered the chamber and lay supine for 5 min on a massage table, with a rapidly inflating blood pressure cuff fitted around the mid-thigh of the right leg (width: 15 cm; Hokanson SC12D, Bellevue, WA, United States). The cuff was connected to a rapid cuff inflator (E20, Hokanson, Bellevue, WA, United States) and supplied by an air compressor. Resting limb blood flow was assessed by rapidly occluding the pressure cuff to 50 mm Hg for 10 s on 2 occasions, each separated by 2 min. Blood flow was determined according to a previous study, whereby a technique for measurement of local muscle  $O_2$  consumption and forearm blood flow in resting and exercising muscle using NIRS and venous occlusion were described<sup>40</sup>

Following LBF assessment, the subjects rested in the chamber on the ergometer (SRM Ergometer, Schoberer Rad Meßtechnik, Jülich, Germany) for ~ 10 min prior to each exercise bout, where they were fitted with a face mask, which supported a turbine volume transducer and gas sampling lines for breath-by-breath measurements of gas flow rates and fractions ( $O_2$  and  $CO_2$ ), respectively. All measurements were performed on a pulmonary gas analyzer (Jaeger Vyntus CPX, Hoechberg, Germany). Pulmonary  $\dot{V}O_2$  ( $\dot{V}O_{2p}$ ),  $\dot{V}CO_2$  and respiratory exchange ratio, minute ventilation ( $\dot{V}_E$ ) and oxygen pulse ( $O_2$ pulse; mL  $\dot{V}O_2$ /heart beat) were recorded for 2 min prior to exercise onset, alongside resting [HHB] and [tHB]. Subjects then transitioned from rest to moderate intensity exercise (external power output = 100 W, < hypoxic gas exchange threshold [GET]) for a 10 min period on the electromagnetically braked cycle ergometer at their *a-priori* fixed self-selected cadence, which was 80 rev·min<sup>-1</sup> for all subjects. Heart rate (HR; Polar FT1, Polar Electro Oy, Kempele, Finland), arterial oxygen saturation (SpO<sub>2</sub> %; Vyntus CPX; CareFusion; Hochberg, Germany) were recorded continuously and reported as the mean of the steady state 10 min exercise bout and during the final 30 s of the ramp protocol (peak SpO<sub>2</sub>).

The  $\dot{V}O_{2p}$  data were initially inspected, with aberrant data points (4 SD of the local mean) removed. These data were then linearly interpolated to 1 s intervals and time aligned, such that  $\dot{V}O_{2p}$  on-kinetics could be modelled from the start of the constant load exercise.

Following baseline correction,  $\dot{V}O_{2p}$  on-kinetics were modelled using a non-linear least squares fitting procedure (Graphpad Prism, Graphpad Software, San Diego, CA). After excluding the first 20 s of data on-transients (ie. phase I<sup>34</sup>), a mono-exponential model was used to characterize all trials. On-transients were modelled based on equation 1:



$$\dot{V}O_{2p}(t) = \dot{V}O_{2pBase} + \dot{V}O_{2pAmp} (1 - e^{-(t-TD)/\tau}) \quad (\text{equation 1})$$

where  $\dot{V}O_{2p}(t)$  = absolute  $\dot{V}O_{2p}$  at a given time  $t$ ;  $\dot{V}O_{2pBase}$  = 2 min baseline resting  $\dot{V}O_2$ ;  $\tau$  = fundamental (phase II) time constant; TD = phase II time delay and  $\dot{V}O_{2pAmp} = \dot{V}O_2$  amplitude (ie.  $\Delta\dot{V}O_{2p} > \dot{V}O_{2pBase}$ ). The data were modelled from 0 s to 240 s, where steady state  $\dot{V}O_{2p}$  ( $\dot{V}O_{2pSS}$ ) was achieved.

The [HHb] derived from the NIRS device was averaged to reduce from 0.1 s to 1 s values and time aligned with the  $\dot{V}O_{2p}$  on-transient data, such that the start of the constant load exercise = 0 s. The time delay for the [HHb] response (TD [HHb]) was determined as the time (s) between the onset of exercise and the point of continual rise above the nadir value. The [HHb] data were modelled using the same mono-exponential curve (equation 1) across 0-120 s of exercise, where steady state was achieved ([HHb]<sub>SS</sub>).<sup>39</sup> The TD [HHb] was included in the model and baseline [HHb] ([HHb]<sub>Base</sub>) was determined from the 2 min pre-exercise resting period.

To evaluate the matching of O<sub>2</sub> delivery and utilization, the corrected  $\dot{V}O_{2p}$  and [HHb] data were normalized relative to their steady state values (ie. % of steady state) and time aligned, such that the start of phase II  $\dot{V}O_{2p}$  kinetics (ie. 20 s) matched the 0 s of [HHb] exercise onset. The normalized data were then averaged into 5 s epochs, with the [HHb]: $\dot{V}O_{2p}$  ratio calculated in series. The mean value from 20 s to 120 s of the exercise transition was

calculated and used to represent the overall O<sub>2</sub> delivery to utilization ratio,<sup>39</sup> where a value of 1.0 represents equal matching and >1.0 indicate mismatching in favour of 'extraction'.

Continuous ramp test (10 W·min<sup>-1</sup>) transition was performed until exhaustion, where time to exhaustion (TTE; min) was determined. Pulmonary  $\dot{V}O_{2\max}$  was determined as the mean value recorded over the final 30 s of the test. Criteria for achieving  $\dot{V}O_{2\max}$  was: [1] reaching volitional exhaustion [2] respiratory exchange ratio > 1.15 and [3] rating of perceived exertion >19 on a 6-20 scale. Breath-by-breath  $\dot{V}O_2$  and  $\dot{V}CO_2$  data from the incremental cycling test was used to plot GET using the simplified v-slope method<sup>37</sup> and evaluation of ventilatory equivalents.<sup>36</sup>

During the baseline and post test steady state trials, the subjects gross efficiency (GE %) was determined as the ratio of work rate accomplished (kcal·min<sup>-1</sup>) to energy expended (kcal·min<sup>-1</sup>). Work rate accomplished was determined as mechanical power (W) × 0.01433. Energy expenditure was calculated from the 2 min respiratory collection between the fourth and sixth min of the 10 min steady state exercise using  $\dot{V}O_2$  and respiratory exchange ratio. The calorific equivalent of O<sub>2</sub> was then determined from the corresponding RER value.<sup>35</sup> Delta efficiency (DE) was determined between the GET and end of the incremental test, as previously reported.<sup>34</sup> Specifically, DE was determined as the ratio of the change in work accomplished/min to the change in metabolic energy expended/min between the VT and end of the incremental test<sup>34</sup>. This was calculated from linear regression ( $y = ax + b$ ) of energy expended/min (y, in kcal/min) and work accomplished/min (x, in kcal/min), where DE is equal to the reciprocal of the slope (1/a).

The subjects lay in a supine position, with automatic inflation cuffs (14.5 cm width, Delfi Medical Innovations, Vancouver, Canada) fitted to the proximal portion of both thighs. In the IPC group, the inflatable cuffs were automatically inflated to the subjects individual limb occlusive pressure, thus ensuring full arterial occlusion. Limb occlusive pressure is automatically recognized by the device, based on the identification of a pulse at each increment of occlusive pressure. When no pulse is consistently recognized, the associated pressure is determined as the LOP by the device. The LOP (mean $\pm$ SD) of the IPC group was 217 $\pm$ 30 mm Hg. The IPC and SHAM protocols were repeated for 7 consecutive days, as reported previously.<sup>20</sup> Specifically, the legs were occluded for 5 min, followed by 5 min reperfusion, which was repeated 4 times (lasting 40 min) in the supine position. The SHAM group were told that they would be receiving the IPC intervention but experienced a lower pressure (20 mm Hg) using the same automatic inflation cuffs. The sum of 8 skinfolds was measured as an indication of whole-body adipose tissue thickness. These included: biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh and an adapted calf measurement at the site of the NIRS optode. Measurements were recorded in duplicate, to the nearest 0.1 mm using skinfold calipers (Harpenden, Burgess Hill, UK). The skin and subcutaneous tissue thickness values (mean $\pm$ SD) for the IPC and SHAM group's calf sites was 9 $\pm$ 2 mm and 8 $\pm$ 2 mm, respectively. This was less than half the distance between source and the detector (35 mm).

### ***Statistical analysis***

Changes in all steady state and incremental test variables and both  $\dot{V}O_2$  and [HHB] on-kinetic profiles were analyzed using 2-way (group [IPC vs Sham] x time [pre vs post]) within and between analyses of variance. Where relevant, assumptions of sphericity were assessed using

Mauchly's test, with any violations adjusted using the Greenhouse-Geisser correction. When significant  $F$ -values were observed, Bonferroni *post-hoc* tests were used to determine differences. Statistical significance was accepted at  $P < 0.05$  for all tests and all analyses were performed on IBM SPSS Statistics (Version 21, IBM Corp., Armonk, NY, USA).

## Results

There were no group x time interactions for TTE ( $F_{(1,12)} = 1.883$ ,  $P = 0.195$ ; Figure 2A), peak SpO<sub>2</sub> ( $F_{(1,12)} = 1.336$ ,  $P = 0.270$ ; Figure 2B) or  $\dot{V}O_{2\max}$  ( $F_{(1,12)} = 0.021$ ,  $P = 0.887$ ; Figure 2C) during the ramp test, nor were main effects of group or time observed for  $\dot{V}O_{2\max}$ , TTE or peak SpO<sub>2</sub> ( $P > 0.05$ ).

\*\*\*Insert fig 2 here\*\*\*

As presented in Table II, there were no group x time interactions for steady state HR ( $F_{(1,12)} = 0.010$ ,  $P = 0.921$ ) or peak HR ( $F_{(1,12)} = 0.027$ ,  $P = 0.872$ ), steady state O<sub>2</sub>pulse ( $F_{(1,12)} = 0.155$ ,  $P = 0.701$ ) or peak O<sub>2</sub>pulse ( $F_{(1,12)} = 0.174$ ,  $P = 0.684$ ). No main effects of group or time were observed for any HR or O<sub>2</sub>pulse variable ( $P > 0.05$ ). Similarly, there was also no group x time interaction for steady state SpO<sub>2</sub> ( $F_{(1,12)} = 3.961$ ,  $P = 0.070$ ), nor were there main group or time effects ( $P > 0.05$ ). There were no interaction effects ( $F_{(1,12)} = 2.593$ ,  $P = 0.133$ ) or main effects of time or group for steady state  $\dot{V}_E$  ( $P > 0.05$ ). Similarly, there were no group x time interaction ( $F_{(1,12)} = 0.013$ ,  $P = 0.932$ ) or time effects ( $P > 0.05$ ) but there was group effects ( $F_{(1,12)} = 4.922$ ,  $P = 0.047$ ) for  $\dot{V}_E$  measured in the final 30 s of the incremental tests, which was consistent across the pre and post trials (Table II).

There were no significant interactions for GE ( $F_{(1,12)} = 2.366$ ,  $P = 0.150$ ) or main effects ( $P > 0.05$ ); however, there were main effects of group ( $F_{(1,12)} = 10.757$ ,  $P = 0.007$ ) and time ( $F_{(1,12)} = 7.188$ ,  $P = 0.020$ ) for DE but no interactions were found ( $P > 0.05$ ). To clarify the reasons for the main effects, in the absence of interactions with time, Bonferroni pairwise comparisons were conducted on the pre to post differences in DE within each group. The IPC group increased DE pre to post intervention ( $P = 0.011$ ), while there were no differences pre to post SHAM intervention ( $P = 0.436$ ). There were no interactions ( $F_{(1,12)} = 2.764$ ,  $P = 0.122$ ) or main effects ( $P > 0.05$ ) for GET (Table II). There were time ( $F_{(1,12)} = 34.231$ ,  $P < 0.001$ ) and group effects ( $F_{(1,12)} = 4.184$ ,  $P = 0.045$ ) only for resting LBF, with higher values in the IPC group compared to SHAM with pre and post intervention values pooled ( $0.4 \pm 0.2$  mL $\cdot$ 100 mg $^{-1}$  $\cdot$ min $^{-1}$  vs.  $0.3 \pm 0.1$  mL $\cdot$ 100 mg $^{-1}$  $\cdot$ min $^{-1}$ , respectively). The LBF results for the IPC group pre and post intervention were  $0.3 \pm 0.1$  and  $0.5 \pm 0.2$  mL $\cdot$ 100 mg $^{-1}$  $\cdot$ min $^{-1}$ , respectively; while the SHAM groups' pre and post intervention results were  $0.2 \pm 0.1$  and  $0.4 \pm 0.1$  mL $\cdot$ 100 mg $^{-1}$  $\cdot$ min $^{-1}$ , respectively.

**\*\*\*Insert Table II here \*\*\***

As presented in Table III, there were group x time interactions for  $\Delta[\text{HHB}]_{\text{ss}}$  ( $F_{(1,12)} = 7.042$ ,  $P = 0.042$ ), with *post-hoc* tests demonstrating lower values ( $P = 0.001$ ) in the post tests compared to pre testing in the IPC group but not SHAM ( $P = 0.180$ ). There were no between-group differences at pre or post testing ( $P > 0.05$ ). The change in [HHB] between the end of the steady state and the end of the ramp test ( $\Delta[\text{HHB}]_{\text{inc}}$ ) did not demonstrate group x time interactions ( $F_{(1,12)} = 0.720$ ,  $P = 0.416$ ) but did have group main effects ( $F_{(1,12)} = 5.917$ ,  $P =$

0.035), with lower values in the IPC group. There were no interactions or main effects found for  $\Delta[tHB]$  during any of the tests ( $P > 0.05$ ).

The  $\dot{V}O_{2p}$  and [HHB] kinetics parameters are presented in Table III. There were no group x time interactions for  $\tau\dot{V}O_{2p}$  ( $F_{(1,12)} = 3.359$ ,  $P = 0.092$ ), TD  $\dot{V}O_{2p}$  ( $F_{(1,12)} = 1.689$ ,  $P = 0.218$ ),  $\dot{V}O_{2pBase}$  ( $F_{(1,12)} = 1.951$ ,  $P = 0.188$ ),  $\dot{V}O_{2pAmp}$  ( $F_{(1,12)} = 0.034$ ,  $P = 0.858$ ),  $\dot{V}O_{2pSS}$  ( $F_{(1,12)} = 2.509$ ,  $P = 0.139$ ). There was a group x time interaction for  $\tau[HHB]$  ( $F_{(1,12)} = 5.660$ ,  $P = 0.045$ ), with *post-hoc* tests revealing slower on-kinetics from pre to post testing in the IPC group ( $P = 0.023$ ) but there were no changes in the SHAM group ( $P = 0.818$ ). However, there were no interactions for TD [HHB] ( $F_{(1,12)} = 0.187$ ,  $P = 0.675$ ), [HHB]<sub>Base</sub> ( $F_{(1,12)} = 0.248$ ,  $P = 0.629$ ), [HHB]<sub>Amp</sub> ( $F_{(1,12)} = 0.041$ ,  $P = 0.844$ ) and [HHB]<sub>SS</sub> ( $F_{(1,12)} = 0.188$ ,  $P = 0.674$ ). There were no group or main effects for all  $\dot{V}O_{2p}$  and [HHB] on-kinetics parameters ( $P > 0.05$ ). The group x time interactions for [HHB]: $\dot{V}O_{2p}$  ( $F_{(1,12)} = 9.086$ ,  $P = 0.013$ ) were the result of reductions from pre to post testing among the IPC group only ( $P < 0.001$ ). There were also lower values in the IPC group compared to SHAM at post testing for [HHB]: $\dot{V}O_{2p}$  ( $P = 0.027$ ). Representative traces of the [HHB]: $\dot{V}O_{2p}$  (panel A) and the corresponding [HHB] (panel B) and  $\dot{V}O_{2p}$  on-kinetics (panel C) are shown in Figure 3. Figure 4 presents a representative trace of the normalized [HHB] and  $\dot{V}O_{2p}$  before (panel B) and after (panel A) the IPC intervention.

\*\*\*Insert fig 3 and 4 here \*\*\*

\*\*\*Insert Table III here\*\*

## Discussion

Seven days of IPC increased DE during the incremental test and reduced  $\Delta[\text{HHB}]$  (reduced  $\text{O}_2$  extraction) of the gastrocnemius compared to the SHAM group across the steady state test. The IPC intervention also reduced the overshoot in  $[\text{HHB}]:\dot{\text{V}}\text{O}_{2\text{p}}$  ratio during exercise onset that was present at baseline among all subjects in severe hypoxia, whereas SHAM did not. Despite this, repeated IPC did not improve  $\dot{\text{V}}\text{O}_{2\text{max}}$  or TTE in severe hypoxia. Similarly, there was no effect on GE during the moderate domain exercise trials, nor was there an alteration in the GET. The effects on DE,  $\Delta[\text{HHB}]$  and  $[\text{HHB}]:\dot{\text{V}}\text{O}_{2\text{p}}$  ratio appeared to be underpinned by an increased limb blood flow, alongside a significant slowing of the  $[\text{HHB}]$  on-transient kinetics, coupled with a modest speeding of the  $\dot{\text{V}}\text{O}_{2\text{p}}$  on-kinetics (although non-significant) in the IPC group. Why these seemingly favourable changes to the muscle-metabolic milieu did not confer any effects on  $\dot{\text{V}}\text{O}_{2\text{max}}$  or exercise tolerance requires further consideration.

Repeated application of IPC across 7 d can improve DE in normoxia, alongside reductions in  $\Delta[\text{HHB}]$  during steady state exercise  $<$  GET, without improving  $\dot{\text{V}}\text{O}_{2\text{max}}$ .<sup>19</sup> We reaffirm these findings in severe hypoxia but in contrast to these previous reports<sup>19</sup> there was no increase in TTE in the current study, despite performing an almost identical incremental test protocol. It is possible that the environmental constraints inhibited the commonly reported ergogenic effect of repeated IPC on exercise tolerance. Such is the severity of exercising in 0.103  $\text{F}_i\text{O}_2$ , it is possible that reduced cerebral  $\text{O}_2$  availability and cortical voluntary activation explained the inability (or reluctance) to continue exercise<sup>13</sup>, despite the improved DE and prior reductions in submaximal muscle  $\text{O}_2$  extraction found here. Of interest, there was no effect of the IPC intervention on  $\Delta[\text{HHB}]$  measured between the end of steady state and end of the

incremental test, indicating that no additional O<sub>2</sub> extraction ‘reserve’ was utilized during the transition to maximal exercise.<sup>18</sup> Thus, in severe hypoxia, submaximal physiological responses appear to be those most affected by repeated IPC.

The only other study to investigate the effect of repeated IPC (5 d) on exercise performance in a hypoxic environment (outdoor altitude of 4342 m; inspired air ~ 142 mm Hg) reported ~ 7% improvements in the time to complete a 12.8 km run compared to placebo.<sup>10</sup> Foster and colleagues also observed concurrent improvements in SpO<sub>2</sub>% (> 5%), which was not found in the present study. In addition, there were no changes in maximal or steady state HR,  $\dot{V}_E$  or  $\dot{V}O_{2p}$ . Collectively, our results indicate that the presumed effect of repeated IPC on pulmonary ventilation (or exercise tolerance)<sup>10</sup> are unlikely to transfer to the simulated (normobaric) hypoxic environment used in the current study. This could be related to previous study designs, where a sufficient period of time without IPC was not provided, as to avoid acute effects on performance.<sup>10</sup> The lack of change in these selected cardio-pulmonary variables likely explains the null effect of IPC on  $\dot{V}O_{2max}$  found here, which is thought to be primarily limited by such factors.<sup>2</sup> The above interpretations concur with reports at lower simulated altitudes following acute IPC interventions ( $F_iO_2 = 0.162$ ;<sup>43</sup>) and infer that the effects of repeated IPC must be realized in other tissues, perhaps more local to the site of interrogation (ie. skeletal muscle<sup>19, 20</sup>).

We have demonstrated improved matching of microvascular O<sub>2</sub> delivery to utilization via the diminished [HHB]: $\dot{V}O_{2p}$  overshoot (~ 0.06) following the IPC intervention during the rest-to-exercise transition. Owing to the environmental constraints imposed in hypoxic environments, which reduces alveolar O<sub>2</sub> tension (PaO<sub>2</sub>) and perturbs O<sub>2</sub> distribution, the



overshoot is pronounced during exercise onsets in hypoxia.<sup>39,46</sup> This is indicative of an increased reliance on  $O_2$  extraction for a given  $\dot{V}O_{2p}$ , inferring a mismatch downstream from the conduit artery between the microvascular  $O_2$  distribution and  $O_2$  utilization.<sup>32</sup> The overshoot magnitude strongly correlates with  $\tau\dot{V}O_{2p}$  when on-kinetics are  $> 20$  s ‘ $O_2$ -dependent’ threshold,<sup>32,39</sup> indicating a delayed metabolic control during exercise. Three weeks of endurance training can diminish the overshoot by a larger magnitude (0.10 to 0.15) and concurrently reduce the  $\tau\dot{V}O_{2p}$  in young and elderly populations,<sup>31</sup> which is interesting in comparison to the current observations, since these were induced after 7 d of passive intervention. In respect to the hypoxic environment, the larger (slower)  $\tau$ [HHB] found after IPC is likely to be related to increased microvascular blood distribution at the site of interrogation.<sup>45</sup> Thus, conversely, the pre intervention overshoot found in all subjects in hypoxia could relate to limb blood flow impairments. The changes post IPC could have partially offset these deficits and corrected the delivery-utilization mismatch.

Heavy-intensity priming exercise has also been reported to elicit similar effects on the transient relationship between [HHB] and  $\dot{V}O_{2p}$  in both normoxic<sup>14</sup> and hypoxic environments,<sup>39</sup> by speeding  $\dot{V}O_{2p}$  kinetics and enhancing the dynamic matching of muscle  $O_2$  extraction and utilization. A 0.06 normalized reduction in the overshoot was reported, which is the same as the magnitude found following repeated IPC in the current study. These effects were ascribed to the increased microvascular blood distribution (rather than bulk flow) observed following heavy priming exercise.<sup>39</sup> It is, therefore, our assumption that the increased resting local blood flow measured during severe hypoxia exposure, which perhaps overcomes the presumed pre exercise reduction in limb blood flow pre exercise<sup>15</sup>, translated to the subsequent exercise transition and supported the observed delivery-utilization

matching. Whilst the post IPC  $\tau\dot{V}O_{2p}$  and  $\dot{V}O_{2Amp}$  were not appreciably different than the pre IPC testing, the subsequent transition to incremental exercise led to improved DE, which indirectly evaluates muscle metabolic efficiency.<sup>27</sup> This might explain why DE, but not GE, was improved after the IPC intervention, as reported previously.<sup>19</sup> The observed adaptations would be beneficial for prolonged, lower intensity exercise tolerance, such as those often performed in high altitude.<sup>28, 38</sup>

The single transitions used in this study to describe the  $\dot{V}O_{2p}$  on-kinetics may have limited the capacity to identify changes in phase II time constant.<sup>1</sup> Regardless, these limitations did not prevent identification of reductions in the overshoot of [HHb]: $\dot{V}O_{2p}$  ratio, and changes in  $\Delta$ [HHb] were also found following the intervention. Secondly, while the techniques used for the IPC and SHAM groups in the current study were typical and subjects were naïve to the intervention, it does not rule out the possibility that the subjects understood the nature of their allocated grouping and subsequent placebo (IPC) effects were present, particularly for voluntary exercise aspects of the study. Similarly, as these subjects were not familiarized to the specific procedures for this study, it is possible that their exercise tolerance was underestimated; however, they all had the same familiarity with laboratory exercise prior to the study. Thirdly, it is possible that the subjects' level of fitness could have confounded the effect of the IPC, given the preliminary evidence that those of higher endurance fitness have been reported to respond to IPC more than less trained<sup>5, 29</sup> but this requires further investigation in repeated IPC applications. Lastly, while the current sample provided sufficient power to detect the anticipated changes in the main outcome variable ( $\Delta$ [HHb]), it is possible that other variables evaluated here were not equally powered. For example, post-hoc power analysis of DE demonstrates that the interaction effects yielded a  $1-\beta$  of 0.49 and, therefore, required a greater sample size to detect a significant interaction.

While 7 d of lower-limb ischemic preconditioning did not improve shorter-term exercise capacity or  $\dot{V}O_{2\max}$  in severe hypoxia, it did positively affect a number of submaximal variables, including reductions in muscle microvascular  $O_2$  extraction and increased control (matching) of the  $O_2$  delivery-utilization relationship during exercise onset. Limb blood flow and slowing of the [HHB] on-transient kinetics might explain these effects but require further investigation. Therefore, those exercising at lower intensities in severe hypoxia could benefit from the improved muscle  $O_2$  efficiency caused by repeated IPC, especially if longer-term exercise bouts are undertaken in severe hypoxic conditions. Improved exercising muscle efficiency and matching of muscle  $O_2$  delivery-utilization during exercise onset, alongside reduced metabolic  $O_2$  demand during steady state exercise, could delay fatigue and improve exercise tolerance during prolonged moderate intensity exercise; however, further investigations are needed to confirm this and corroborate these preliminary data.

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**Tables:****Table I.** Baseline participant characteristics of the ischemic preconditioning and SHAM groups.

	IPC ( <i>n</i> = 7)	SHAM ( <i>n</i> = 7)
Body mass (kg)	86.0 ± 13.3	80.1 ± 10.8
Stature (cm)	181 ± 7	181 ± 9
Sum of 8 skinfolds (mm)	81 ± 6	79 ± 16
Age (y)	27 ± 3	26 ± 4
Baseline $\dot{V}O_{2\max}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	32.2 ± 2.9	30.8 ± 3.0

Note: *t*-tests demonstrated no differences between groups for any variable (*P* > 0.05).

**Table II.** Heart rate (HR), oxygen pulse (O<sub>2</sub>pulse), mean arterial oxygen saturation (SpO<sub>2</sub>) gross efficiency (GE), minute ventilation ( $\dot{V}_E$ ) delta efficiency (DE) and gas exchange threshold (GET) during steady state and incremental exercise (mean  $\pm$  SD), pre and post ischemic preconditioning (IPC) or SHAM interventions.

	Pre		Post	
	IPC (n = 7)	SHAM (n = 7)	IPC (n = 7)	SHAM (n = 7)
<b>Steady state</b>				
Mean HR (beats·min <sup>-1</sup> )	133 $\pm$ 13	147 $\pm$ 12	131 $\pm$ 15	145 $\pm$ 14
Mean O <sub>2</sub> pulse (mL·beat <sup>-1</sup> )	14 $\pm$ 1	12 $\pm$ 2	14 $\pm$ 1	13 $\pm$ 2
GE (%)	16 $\pm$ 1	17 $\pm$ 3	16 $\pm$ 1	16 $\pm$ 1
SpO <sub>2</sub> (%)	68 $\pm$ 3	72 $\pm$ 5	71 $\pm$ 4	71 $\pm$ 6
$\dot{V}_E$ (L·min <sup>-1</sup> )	46.7 $\pm$ 5.9	50.4 $\pm$ 6.9	48.1 $\pm$ 4.7	48.3 $\pm$ 5.5
<b>Incremental test</b>				
Peak HR (beats·min <sup>-1</sup> )	173 $\pm$ 8	175 $\pm$ 12	170 $\pm$ 9	174 $\pm$ 12
Peak O <sub>2</sub> pulse (mL·beat <sup>-1</sup> )	16 $\pm$ 3	14 $\pm$ 2	16 $\pm$ 3	14 $\pm$ 2
GET (%)	73 $\pm$ 6	73 $\pm$ 8	77 $\pm$ 6	73 $\pm$ 7
DE (%) <sup>a,b</sup>	34 $\pm$ 2	31 $\pm$ 2	39 $\pm$ 6	32 $\pm$ 3
$\dot{V}_E$ (L·min <sup>-1</sup> ) <sup>a</sup>	113.4 $\pm$ 23.7	99.1 $\pm$ 12.9	113.4 $\pm$ 25.4	100.7 $\pm$ 17.4

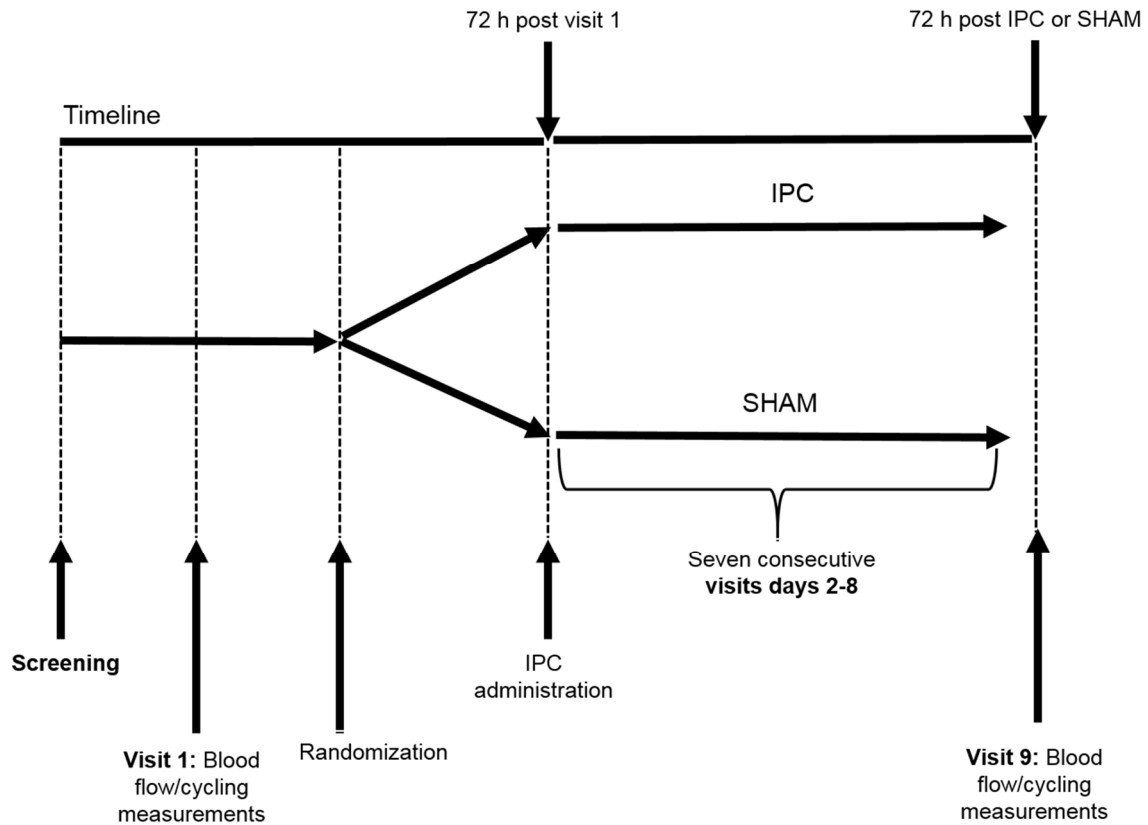
Note: a = main effect of group; b = main effect of time ( $P < 0.05$ ).

**Table III.** The deoxyhemoglobin concentration [HHb] and pulmonary oxygen uptake responses ( $\dot{V}O_{2p}$ ) from rest to moderate exercise (mean $\pm$ SD), pre and post ischemic preconditioning (IPC) or SHAM interventions.

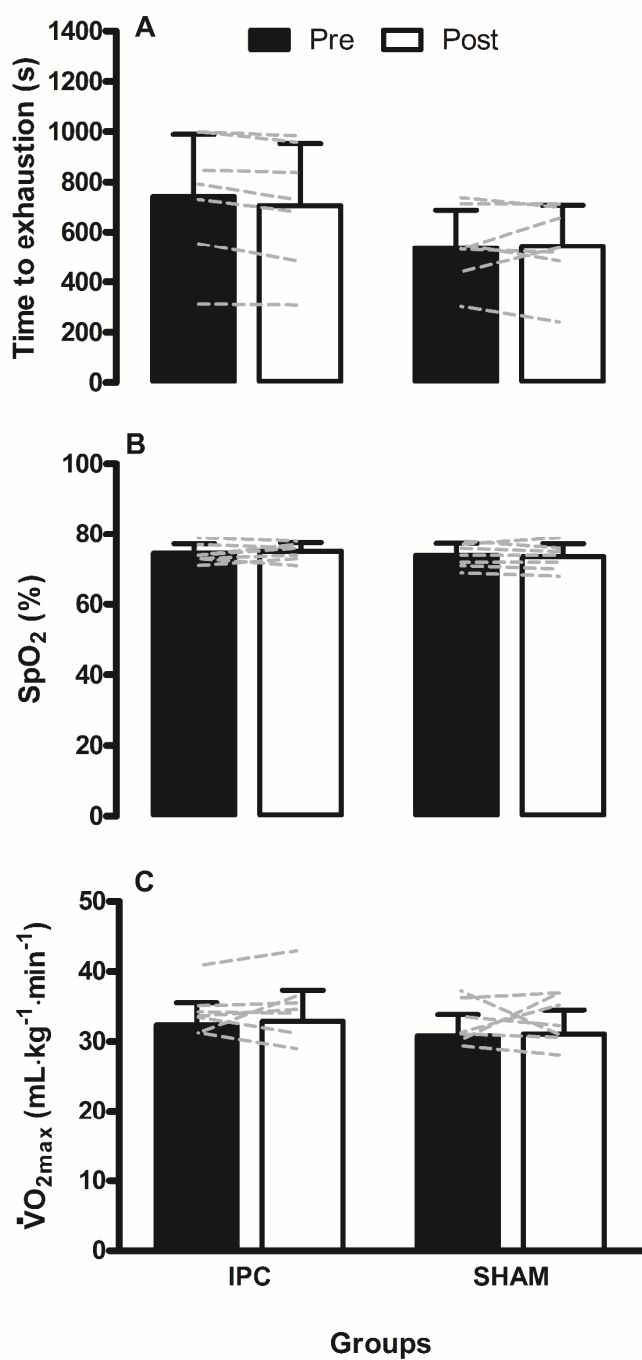
	Pre		Post	
	IPC ( <i>n</i> = 7)	SHAM ( <i>n</i> = 7)	IPC ( <i>n</i> = 7)	SHAM ( <i>n</i> = 7)
<b><i>Pulmonary measures</i></b>				
$\dot{V}O_{2pBase}$ (mL)	680 ± 220	583 ± 118	556 ± 216	694 ± 98
$\dot{V}O_{2pAmp}$ (mL)	1237 ± 122	1139 ± 190	1271 ± 250	1156 ± 135
$\dot{V}O_{2pSS}$ (mL)	1917 ± 267	1723 ± 279	1827 ± 100	1850 ± 155
$\tau\dot{V}O_{2p}$ (s)	28.5 ± 4.5	28.3 ± 3.6	23.5 ± 2.4	29.0 ± 7.1
TD $\dot{V}O_{2p}$ (s)	15.1 ± 5.1	11.3 ± 4.2	14.5 ± 3.4	16.2 ± 6.1
<b><i>Microvascular measures</i></b>				
[HHB] <sub>Base</sub> (μM)	4.8 ± 2.8	2.9 ± 2.4	5.8 ± 3.2	3.4 ± 2.2
[HHB] <sub>Amp</sub> (μM)	15.8 ± 5.3	18.2 ± 8.2	14.0 ± 4.2	17.3 ± 8.9
[HHB] <sub>SS</sub> (μM)	18.8 ± 2.9	24.7 ± 9.2	17.8 ± 3.1	26.3 ± 14.1
$\tau$ [HHB] (s) <sup>c</sup>	8.8 ± 3.4*	8.6 ± 3.5	11.7 ± 5.3	8.4 ± 2.5
TD [HHB] (s)	4.8 ± 2.8	2.9 ± 2.4	5.9 ± 3.2	3.4 ± 2.1
[HHB] : $\dot{V}O_{2p}$ <sup>c</sup>	1.10 ± 0.04*	1.11 ± 0.02	1.04 ± 0.05	1.09 ± 0.02 <sup>†</sup>
$\Delta$ [HHB] <sub>SS</sub> <sup>a,b,c</sup>	17.3 ± 5.3*	18.8 ± 3.3	14.0 ± 4.9	17.8 ± 3.9
$\Delta$ [HHB] <sub>inc</sub> <sup>a</sup>	5.9 ± 1.2	4.8 ± 1.2	5.8 ± 2.2	3.7 ± 1.4
$\Delta$ [tHB] <sub>SS</sub>	2.9 ± 2.8	3.8 ± 1.5	4.5 ± 2.1	3.2 ± 0.7
$\Delta$ [tHB] <sub>inc</sub>	4.5 ± 3.5	4.3 ± 2.4	3.6 ± 2.1	4.5 ± 1.1

Note: a = main effect of group; b = main effect of time; c = interaction effect ( $P < 0.05$ ). \* = different from post-test comparison of that group ( $P < 0.05$ ); † = different from IPC at post test. All measurements of muscle microvascular relative concentrations were measured (μM) using a spatially resolved spectrometer. Delta values, denoted by  $\Delta$ , pertain to change scores between 30 s epochs pre and end stage.

## Figures

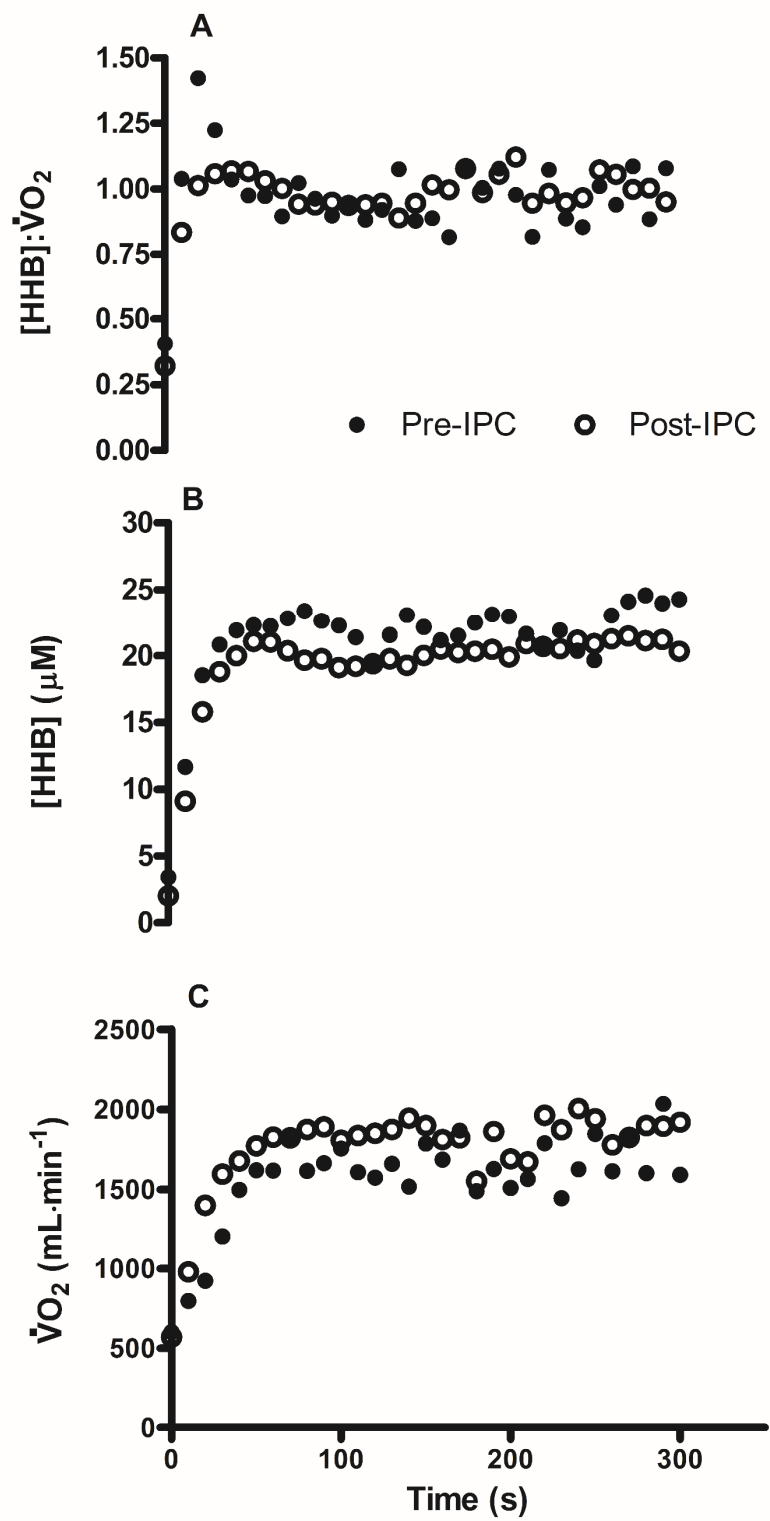


**Figure 1.** Schematic diagram of the experimental timeline. Screening included written informed consent of subjects and body composition analysis. Visits 1 and 9 included resting limb blood flow and cycling assessment, comprising a steady state cycling assessment at 100 W and step test to exhaustion at 10 W increments. Visits 2-8: Group A = ischemic preconditioning (IPC) at 100% limb occlusive pressure; Group B = SHAM at 20 mm Hg. IPC administration = IPC of the lower extremities consisted of 5 min occlusion, followed by 5 min reperfusion, repeated four times (40 min protocol; 20 min total ischemia).

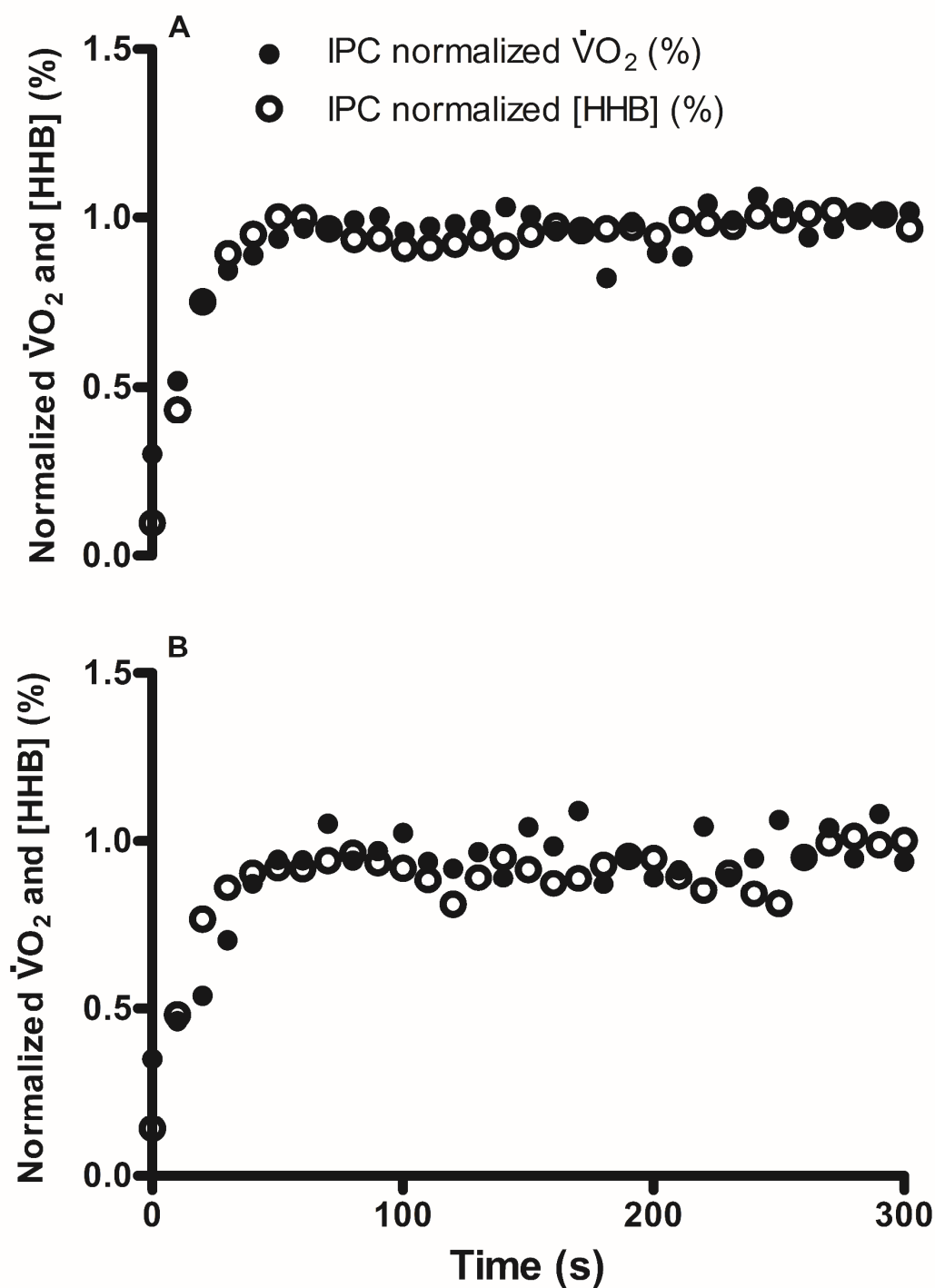


**Figure 2.** Maximal oxygen consumption ( $\dot{V}O_{2max}$ ), time to exhaustion (TTE) and final arterial oxygen saturation (SpO<sub>2</sub> %) during the incremental ramp test (mean±SD) pre and post ischemic preconditioning (IPC;  $n = 7$ ) or SHAM ( $n = 7$ ) interventions. Error bars are SD.





**Figure 3.** Representative traces of the deoxyhemoglobin to pulmonary oxygen uptake ratio ( $[HHb]:\dot{V}O_{2p}$ ) (panel A) and the corresponding  $[HHb]$  (panel B) and  $\dot{V}O_{2p}$  on-kinetics (panel C) during the rest to moderate exercise transition in a subject in the ischemic preconditioning (IPC) group from pre-to-post intervention. Data are presented in 10 s intervals. Error bars are SD.



**Figure 4.** Representative traces of the normalized pulmonary oxygen uptake ( $\dot{V}O_{2p}$ ) and deoxyhemoglobin ([HHB]) pre (panel A) and post (panel B) in a subject in the ischemic preconditioning (IPC). Data are presented in 10 s intervals. Error bars are SD.