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RESEARCH ARTICLE

Ischaemic preconditioning blunts exercise-induced mitochondrial dysfunction, speeds oxygen uptake kinetics but does not alter severe-intensity exercise capacity

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Abstract

We examined the effect of ischaemic preconditioning (IPC) on severe-intensity exercise performance, pulmonary oxygen uptake ($\dot{V}_{O_2}$) kinetics, skeletal muscle oxygenation (muscle tissue O_2 saturation index) and mitochondrial respiration. Eight men underwent contralateral IPC (4 × 5 min at 220 mmHg) or sham-control (SHAM; 20 mmHg) before performing a cycling time-to-exhaustion test (92% maximum aerobic power). Muscle (vastus lateralis) biopsies were obtained before IPC or SHAM and ~1.5 min postexercise. The time to exhaustion did not differ between SHAM and IPC (249 ± 37 vs. 240 ± 32 s; $P = 0.62$). Pre- and postexercise ADP-stimulated (P) and maximal (E) mitochondrial respiration through protein complexes (C) I, II and IV did not differ ($P > 0.05$). Complex I leak respiration was greater postexercise compared with baseline in SHAM, but not in IPC, when normalized to wet mass ($P = 0.01$ vs. $P = 0.19$), mitochondrial content (citrate synthase activity, $P = 0.003$ vs. $P = 0.16$; CI+IIP, $P = 0.03$ vs. $P = 0.23$) and expressed relative to P ($P = 0.006$ vs. $P = 0.30$) and E ($P = 0.004$ vs. $P = 0.26$). The $\dot{V}_{O_2}$ mean response time was faster (51.3 ± 15.5 vs. 63.7 ± 14.5 s; $P = 0.003$), with a smaller slow component (270 ± 105 vs. 377 ± 188 ml min^{-1}; $P = 0.03$), in IPC compared with SHAM. The muscle tissue O_2 saturation index did not differ between trials ($P > 0.05$). Ischaemic preconditioning expedited $\dot{V}_{O_2}$ kinetics and appeared to prevent an increase in leak respiration through CI, when expressed proportional to E and P evoked by severe-intensity exercise, but did not improve exercise performance.

KEYWORDS
ischaemia, oxidative metabolism, performance

1 | INTRODUCTION

Various pre-exercise strategies are adopted by athletes to improve exercise performance (Austin & Seebohar, 2011; Kilduff et al., 2013). One such strategy is the application of ischaemic preconditioning (IPC), which consists of brief, repeated bouts of blood-flow occlusion followed by reperfusion. Although initially demonstrated to confer cardioprotective effects in a clinical setting (Murry et al., 1986; Pang et al., 1995), there is evidence to suggest that IPC can improve performance during cycling (Cruz et al., 2015, 2016; De Groot et al., 2010; Kido et al., 2015), running (T. G. Bailey, Jones et al., 2012) and swimming (Jean-St-Michel et al., 2011) exercise. However, the effect of IPC on exercise performance is equivocal, with some studies indicating that IPC does not improve high-intensity intermittent running performance (I. C. Marocolo et al., 2017) or running economy (Kaur et al., 2017) and might even evoke an ergolytic effect on power output during maximal sprint cycling (Paixão et al., 2014). Recent systematic reviews and meta-analyses examining the effect of IPC on exercise performance have also presented conflicting conclusions (Incognito et al., 2016; M. Marocolo et al., 2016; Salvador et al., 2016). Furthermore, these systematic reviews have highlighted a paucity of robust research assessing the ergogenic potential of IPC for severe-intensity exercise, which encompasses events ranging from ~3 to 20 min in duration (Poole et al., 1988).

In addition to equivocal effects on exercise performance, the putative underpinning physiological mechanisms by which IPC administration might be ergogenic are unclear. The existing evidence suggests that peripheral vascular function, tissue O_2 delivery and extraction and mitochondrial function can be improved by IPC (Salvador et al., 2016). Indeed, IPC has been reported to improve O_2 delivery and to promote endothelial functional sympatholysis in response to sympathetic activation, at rest and during handgrip exercise (Horiuchi et al., 2015). Ischaemic preconditioning has also been demonstrated to attenuate the declines in blood flow after severe-intensity running exercise (T. G. Bailey, Birk et al., 2012). However, the finding of improved muscle tissue O_2 delivery after IPC is inconsistent, with no changes in these parameters observed during handgrip exercise (Barbosa et al., 2015). It has been reported that IPC can increase voluntary force production during maximal contractions of the knee extensors in the absence of muscle hyperaemia [inferred from unaltered near-infrared spectroscopy (NIRS)-derived muscle total haemoglobin + myoglobin], but concomitant with increased muscle O_2 extraction (inferred from increased NIRS-derived deoxygenated haemoglobin + deoxygenated myoglobin) (Paradis-Deschênes et al., 2016). With regard to whole-body exercise, although Turnes et al. (2018) did not observe any changes in performance during a 2 km rowing time trial, the muscle tissue O_2 saturation index (TSI) was lowered, without a change in pulmonary oxygen uptake ($\dot{V}_{O_2}$), suggesting increased muscle O_2 extraction after administration of IPC. Although it has been reported that IPC can speed $\dot{V}_{O_2}$ kinetics after the onset of exercise (Kido et al., 2015; Kilding et al., 2018), $\dot{V}_{O_2}$ kinetics were assessed during moderate- and heavy-intensity step exercise tests of a fixed duration. Although Kilding et al. (2018) did report small improvements in a separate 4 km time-trial performance after IPC, $\dot{V}_{O_2}$ kinetics were not examined. Therefore, the extent to which IPC might improve the performance of severe-intensity exercise through enhanced $\dot{V}_{O_2}$ kinetics has yet to be determined directly.

Early clinical investigations demonstrated that IPC improved O_2 utilization and better maintained intramuscular pH, muscle ATP and phosphocreatine content in animal cardiac muscle (Kida et al., 1991; Murry et al., 1986; Pang et al., 1995), a finding that has been linked to the maintenance of mitochondrial respiratory function after ischaemia (Andreas et al., 2011; Thaveau et al., 2007). Specifically, IPC has been shown to preserve mitochondrial coupling efficiency, via reduced proton leak, in isolated mitochondria from cardiac muscle in animal models (Nadtochiy et al., 2006; Quarrie et al., 2011). These studies demonstrated that IPC attenuated large increases in leak respiration after prolonged bouts of ischaemia. There is evidence demonstrating decreased ADP-stimulated respiration after a 5 km cycling time trial (Layec et al., 2018) and increased uncoupled leak respiration after continuous, high-intensity interval and sprint interval exercise (Trewin et al., 2018). However, the extent to which IPC might offset these exercise-induced perturbations to mitochondrial respiratory capacity has yet to be determined. Moreover, research has demonstrated that several parameters of mitochondrial respiration, determined using high-resolution respirometry, are positively associated with faster $\dot{V}_{O_2}$ kinetics (Christensen et al., 2016), suggesting that an interaction between these variables might underpin the physiological mechanisms of IPC.

New Findings

- **What is the central question of this study?**
  Ischaemic preconditioning is a novel pre-exercise priming strategy. We asked whether ischaemic preconditioning would alter mitochondrial respiratory function and pulmonary oxygen uptake kinetics and improve severe-intensity exercise performance.
- **What is the main finding and its importance?**
  Ischaemic preconditioning expedited overall pulmonary oxygen uptake kinetics and appeared to prevent an increase in leak respiration, proportional to maximal electron transfer system and ADP-stimulated respiration, that was evoked by severe-intensity exercise in sham-control conditions. However, severe-intensity exercise performance was not improved. The results do not support ischaemic preconditioning as a pre-exercise strategy to improve exercise performance in recreationally active participants.

The primary aim of this study was to investigate the effect of IPC on time to exhaustion (TTE) during severe-intensity cycling, compared with sham-control conditions (SHAM). It was hypothesized that IPC would improve the performance of severe-intensity exercise. Furthermore, in this study we aimed to examine the effects of IPC on mitochondrial function and $\dot{V}_{O_2}$ kinetics. It was hypothesized that mitochondrial coupling efficiency would be reduced, owing to increased leak respiration immediately postexercise and that IPC would maintain respiratory coupling effiency by attenuating increases in leak respiration; IPC would increase postexercise ADP-coupled mitochondrial respiration and, subsequently, speed overall $\dot{V}_{O_2}$ kinetics.

2 | METHODS

2.1 | Ethical approval

All experimental procedures were approved by the Loughborough University Ethics Approvals Human Participants Sub-Committee (R19-P138) and conformed to the *Declaration of Helsinki*, except for registration in a database. Participants were fully informed of the risks and discomforts associated with all experimental trials before providing written, informed consent.

2.2 | Participants

Eight healthy, recreationally active men (mean ± SD age 23 ± 2 years, height 1.78 ± 0.07 m and body mass 79 ± 7 kg) volunteered to participate in this study. All completed health and biopsy screening questionnaires before participation to mitigate for contra-indications to maximal exercise, muscle biopsy procedures and IPC. Participants did not have a history of neuromuscular, haematological or musculoskeletal abnormalities and were not using dietary or pharmacological interventions during the study period.

2.3 | Experimental design

After preliminary testing and familiarization, in a repeated-measures, counterbalanced crossover design, participants performed two TTE tests on a cycle ergometer on separate occasions, once after IPC and once after SHAM. Each trial was separated by ≥7 days. On each occasion, muscle biopsies were obtained before IPC/SHAM and ~1.5 min after the TTE test. Pulmonary gas exchange, heart rate (HR) and muscle oxygenation were measured throughout the TTE test.

All exercise tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, The Netherlands). Experiments were conducted in the same environmentally controlled laboratory (19–21°C and 40–50% humidity). Participants were required to arrive at the laboratory (at 09.30 h ±1 h to limit the effects of circadian variation) in a rested and fully hydrated state, after an overnight fast and having eaten a standardized breakfast of 77 g of porridge oats with 490 ml semi-skimmed milk. Participants were required to refrain from strenuous physical activity in the 48 h before, and from alcohol and caffeine 24 h before, each experimental session.

2.4 | Preliminary and familiarization sessions

Participants attended two preliminary sessions, with the second ≥7 days before the first experimental trial. During the first visit, anthropometric measures were obtained, and participants completed a ramp incremental cycling test to exhaustion to establish peak oxygen uptake ($\dot{V}_{O_2peak}$) and maximal aerobic power (MAP). Participants began to cycle at a freely chosen, constant pedal cadence, at 25 W. Power output was increased linearly at a rate of 25 W min^{-1} until volitional exhaustion or when pedal cadence fell 10% below the freely chosen cadence for >5 s, despite strong verbal encouragement. Pulmonary gas exchange was measured continuously throughout exercise. The $\dot{V}_{O_2peak}$ and MAP were defined as the highest $\dot{V}_{O_2}$, determined from a 5 s rolling average, over a 30 s period and power output averaged over the final 60 s of the test, respectively. After a 30 min rest, participants were familiarized with the TTE test, which was performed at 92% MAP, and designed to elicit an exercise duration of ~4 min.

During the second visit, participants were familiarized with the IPC and SHAM protocols, in which they were subjected to 2 × 5 min contralateral occlusion and reperfusion cycles with the cuff inflated to the IPC (220 mmHg) and SHAM (20 mmHg) pressures. After 30 min rest, participants were again familiarized with the TTE test at 92% MAP. If the TTE differed from the ~4 min target duration by a substantial amount (e.g., 1 min), the relative intensity was adjusted for the subsequent experimental trials. This occurred in two participants, for whom the intensity was reduced to 90% MAP.

2.5 | Experimental protocol

On arrival at the laboratory, participants initially rested in a supine position whilst the portable NIRS device (Portamon; Artinis Medical Systems, Zetten, The Netherlands) and HR monitor (M400; Polar Electro, Helsinki, Finland) were positioned. After another 15 min rest, a muscle biopsy was obtained. Resting TSI was then determined during a 10 min period. Next, the occlusion cuffs (SC12L; Hokanson, Bellevue, USA) were fitted, and the experimental IPC or SHAM procedure was completed. Participants then rested in a semi-supine position for 60 min, during which the site for the postexercise muscle biopsy was prepared and covered with sterile gauze. Approximately 55 min after the IPC/SHAM procedure, the participant mounted the cycle ergometer and the facemask for pulmonary gas exchange was fitted in order to begin the TTE test protocol, ensuring that the start of the exercise was precisely 60 min after the end of the IPC/SHAM procedure. At the cessation of exercise, participants quickly

dismounted the cycle ergometer, and a muscle biopsy was obtained within 1.5 min.

2.6 | Ischaemic preconditioning/SHAM procedure

Automatic occlusion cuffs were positioned as proximally as possible around both thighs. In the IPC conditions, cuffs were inflated (E20 Rapid cuff inflator and AG101 Cuff Inflator Air Source, Hokanson, Bellevue, USA) to 220 mmHg for 5 min, followed by 5 min of reperfusion, in a contralateral fashion. This procedure was repeated four times, for a total duration of 40 min. This IPC protocol was used because it is in line with most of the literature that has demonstrated improved exercise performance (Salvador et al., 2016). In the SHAM conditions, the same inflation procedure was used, but the cuffs were inflated to 20 mmHg, which is an insufficient pressure to occlude blood flow. In an attempt to overcome the inherent difficulties of blinding participants to IPC, it was suggested that both interventions might improve exercise capacity and that the SHAM conditions resulted in partial, venous occlusion. Participants were not informed of this until the completion of both trials.

2.7 | Time-to-exhaustion test protocol

The TTE trial began with a 3 min period of seated rest on the cycle ergometer before completion of 2 min of cycling at 20 W; the final 60 s obtained was averaged and represented baseline $\dot{V}_{O_2}$. Resistance was then applied instantaneously to elicit a power output equivalent to ~92% MAP (as determined during the $\dot{V}_{O_2peak}$ test and subsequent familiarization sessions). Participants were instructed to increase the pedal cadence to a self-selected pace in the 5 s before the beginning of the test phase. Exercise was continued until volitional exhaustion or when pedal cadence fell 10% below the freely chosen cadence for >5 s, despite strong verbal encouragement. Standardized verbal encouragement was provided by two investigators throughout all testing. Participants were blinded from any time feedback during the test. Pulmonary gas exchange was measured continuously throughout the test protocol. Heart rate was measured every minute and rating of perceived exertion (RPE; 6–20) at 2 min ± 15 s (deliberately randomized to ensure blinding to the elapsed time).

2.8 | Muscle biopsy sampling and analysis

Muscle biopsies were obtained under local anaesthesia (1% lidocaine) from the medial portion of the vastus lateralis, using the micro-biopsy technique (Acecut 11-gauge biopsy needle; TSK, Tokyo, Japan). Muscle biopsies at each time point were obtained through separate incisions on the same leg, ~2 cm apart. Two samples (~30 mg each) were taken from the respective incision at each time point. A small (~8 mg) section of the first sample from each time point was immediately placed in BioPS solution (see section 2.9) and stored on ice for analysis by high-resolution respirometry within 8 h. The remainder of this sample and the second sample were immediately snap-frozen in liquid nitrogen and stored at −80°C for future analysis of citrate synthase (CS) activity, a valid marker of mitochondrial content (Larsen et al., 2012).

2.9 | High-resolution respirometry

Muscle samples were placed in ice-cold BioPS (mM: 2.77 CaK_2EGTA, 7.23 K_2EGTA, 5.77 Na_2ATP, 6.56 $MgCl_2$, 20 taurine, 50 2-(*N*-morpholino)ethanesulfonic acid, 15 Na_2-phosphocreatine, 20 imidazole and 0.5 dithiothreitol, adjusted to pH 7.1 by titration with KOH). Under a low-power microscope, muscle samples were dissected free of connective tissue and fat before mechanical separation of two muscle fibre bundles, each weighing 1–3 mg. Subsequently, the muscle fibres were quickly transferred into an ice-cold saponin solution [50 μg/ml BioPS, 20 μl saponin stock (5 mg saponin/ml BioPS)] and gently agitated on ice for 30 min, in order to facilitate chemical permeabilization of the plasma membrane. The samples were then transferred into 2 ml of MiR05 (a respiration medium, mM: 110 sucrose, 60 K^+-lactobionate, 0.5 EGTA, 3 $MgCl_2$, 20 taurine, 10 KH_2PO_4 and 20 Hepes, adjusted to pH 7.1 with KOH at 37°C and 1 g/L bovine serum albumin, essentially fatty-acid free) and gently agitated on ice for 10 min, before being transferred into a fresh 2 ml of MiR05 to ensure that no saponin remained in the bundles or media (Pesta & Gnaiger, 2012). The muscle fibres were then dried for 5 s on filter paper, weighed and transferred into a fresh 1 ml of ice-cold MiR05. Mitochondrial respiration was measured in duplicate after fully immersing the fibres in MiR05 at 37°C in the chamber of a high-resolution respirometer (O_2k; Oroboros, Innsbruck, Austria). Using DatLab v.7.3 software (Oroboros, Innsbruck, Austria), the O_2 concentration (in nanomoles per millilitre) and flux (in picomoles per second per milligram) were recorded instantaneously. To avoid any potential O_2 diffusion limitation, the O_2 concentration was maintained in the range of 200–500 μM (Pesta & Gnaiger, 2012). This required monitoring the O_2 concentration and reoxygenating by direct syringe injection of pure O_2 when necessary.

A substrate–uncoupler–inhibitor–titration protocol (SUIT 8) was used to determine leak respiration, ADP-coupled oxidative phosphorylation (OXPHOS) and electron transfer system (ETS) capacity (E) in mitochondrial protein complexes I–IV (CI–CIV). Steady states of O_2 flux were marked on DatLab v.7.3 (Oroboros, Innsbruck, Austria) after titrations in the following sequence. First, 5 mM pyruvate and 2 mM malate were added in the absence of adenylates to measure leak respiration through CI (CIL). Second, to ensure saturating concentrations of ADP, 5 mM was titrated, before multiple titrations of 2.5 mM of ADP until respiration ceased to increase. Third, 10 mM of glutamate was added to determine maximal OXSPHOS capacity through CI (CIP). Fourth, 10 μM of cytochrome *c* was added to test the integrity of the outer mitochondrial membrane, which was accepted as an increase in respiration of <15%. Fifth, 10 mM succinate was added and, after stabilization, repeated titrations of 2.5 mM ADP were administered until respiration ceased to increase, ensuring saturating

concentrations through the succinate-linked respiratory pathway. The maximal steady-state respiration after this step represents measurement of OXSPHOS through CI and CII (CI+IIP). Sixth, a series of stepwise carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) titrations (0.75–1.5 mM) were added, until no further increase in O_2 flux was observed, for assessment of maximal ETS capacity through CI and CII (CI+IIE). Seventh, 0.5 μM rotenone, a CI inhibitor, was added for the determination of E through CII alone (CIIE). Eighth, addition of 2.5 μM antimycin A, a CIII inhibitor, allowed for the measurement of, and latterly correction for, residual O_2 consumption (ROX), indicative of non-mitochondrial O_2 consumption. Ninth, artificial electron donors for CIV, 2 mM ascorbate and 0.5 mM *N*,*N*,*N*′,*N*′- tetramethyl-*p*-phenylenediamine (TMPD), were added to measure E through CIV (CIVE). Finally, ≥100 mM of sodium azide was added, inhibiting all mitochondrial respiration and allowing for the calculation of autoxidation of the O_2k electrode, which is artificially increased after the titration of ascorbate and TMPD, to allow for the correction of CIVE. Chamber cleaning procedures were followed strictly before and after all analysis according to the recommendations of the manufacturer (Oroboros, Innsbruck, Austria) to ensure accuracy and reliability.

For CS activity, ~15–50 mg of frozen muscle tissue was homogenized in cold lysis buffer (1:10 wet weight/volume) containing PBS, 0.2% Triton X-100, 1 mM EDTA and protease and phosphatase inhibitor cocktail (Fisher Scientific, Loughborough, UK). Samples were blitzed using a tissue lyser (Qiagen, Manchester, UK) for 4 min at 20 Hz and centrifuged at 12,000*g* for 10 min to pellet insoluble material. The supernatant was then transferred to a fresh tube and protein concentration determined in duplicate by Pierce 660 protein assay according to the manufacturer's instructions (Fisher Scientific, Loughborough, UK). The CS activity was analysed in triplicate on a 96-well plate. Ten microlitres of muscle homogenate (1 mg ml^{-1}) was titrated into each well, which contained 40 μl of 3 mM acetyl CoA, 25 μl of 1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) solution to 165 μl of 100 mM Tris buffer (pH 8.3) and 10 μl of 1% Triton X-100. Immediately, before placing the plate into the spectrophotometer (Varioskan Flash; Thermo Fisher Scientific, Loughborough, UK), 15 μl of 10 mM oxaloacetic acid was added to the wells. Samples were maintained at 30°C and, after 30 s of linear agitation, absorbance at 412 nm was recorded every 15 s for 3 min. Values were corrected for the path length of the plate and were expressed in moles per hour per kilogram. The coefficient of variation for CS activity was 8.4 ± 5.7%.

Respirometry values were corrected to CS activity, and these values were used to calculate flux control ratios (FCRs) for each time point in each of the experimental conditions, briefly: the leak control ratio (LCR), the quotient of CIL over CI+IIE; the phosphorylation control ratio (PCR), the quotient of CI+IIP over CI+IIE; the coupling or inverse respiratory control ratio (InvRCR), the quotient of CIL over CI+IIP; the substrate control ratio (SCR), the quotient of CIP over CI+IIP at constant OXPHOS; and reserve CIV capacity (CIVres), the quotient of CI+IIP over CIVE. Respirometry values were also corrected to CI+IIP.

2.10 | Pulmonary gas exchange and $\dot{V}_{O_2}$ kinetics

Pulmonary gas exchange was continuously sampled during exercise tests (Vyntus CPX; Carefusion, San Diego, California, USA). The system was calibrated before testing using gas references of a known composition (15.98% O_2; 5% CO_2) and a 3 L syringe (Carefusion, San Diego, California, US). Participants were fitted with a facemask and one-way valve attachment (7450 V2 Series; Hans Rudolph, Shawnee, Kansas, USA) for continuous breath-by-breath assessment of $\dot{V}_{O_2}$ and CO_2 production. Breath-by-breath $\dot{V}_{O_2}$ data from each trial were treated as described previously (Bailey et al., 2011). A single-exponential model, without time delay, with the fitting window commencing at $t = 0$ s [equivalent to the mean response time (MRT)], was used to characterize the kinetics of the overall $\dot{V}_{O_2}$ response during the trials as described in the equation:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2}\text{baseline} + A(1 - e^{-(t/\text{MRT})}), \quad (1)$$

where $\dot{V}_{O_2}(t)$ represents the absolute $\dot{V}_{O_2}$ at a given time *t*, $\dot{V}_{O_2}$ baseline represents the mean $\dot{V}_{O_2}$ over the final 90 s of baseline measurements, and *A* and MRT represent the amplitude and mean response time, respectively, describing the overall increase in $\dot{V}_{O_2}$ above baseline. The $\dot{V}_{O_2}$ MRT was quantified with the fitting window constrained to both completion time (end-exercise) for each participant in both the IPC and SHAM conditions and an isotime of 210 s. The absolute $\dot{V}_{O_2}$ at 120 s, 210 s (±5 s) and end-exercise (average over the final 10 s) were calculated. The $\dot{V}_{O_2}$ slow component amplitude was estimated by subtracting $\dot{V}_{O_2}$ uptake at 120 s from the end of exercise.

2.11 | Near-infrared spectroscopy

The NIRS device (Portamon, Artinis, Utrecht, The Netherlands) was attached to the opposite leg from which the muscle biopsy was obtained, with the same leg used for both trials and counterbalanced for limb dominance between participants. Initially, femur length was determined (defined as the distance from the anterior femoral head to the proximal patella border), and a mark was made at 55% of this length on the most lateral point of the vastus lateralis. Skinfold thickness (Harpenden skinfold callipers; British Indicators, UK) was measured at this site to ensure that the distance was within the range of photon transmission (20 mm). The area around this mark was cleaned with an alcohol wipe and shaved. The NIRS device was then attached to the leg and covered using kinesiology tape. Participants were provided with cycling shorts, which were pulled over the device to prevent environmental light from contaminating the NIRS signal. The NIRS device omits near-infrared light from three diodes at 760 and 850 nm, at distances of 30, 35 and 40 mm from the receiver. Data were sampled at 10 Hz and recorded with Oxysoft software (Artinins, Utrecht, The Netherlands). During offline analysis, data were exported and averaged to 1 s intervals. The TSI (as a percentage) was calculated internally by

the NIRS device, as described in the equation:

$$\mathrm{TSI} = \frac{(\mathrm{OxyHb} + \mathrm{Mb})}{(\mathrm{OxyHb} + \mathrm{Mb}) + (\mathrm{DeoxyHb} + \mathrm{Mb})} \times 100 \quad (2)$$

where OxyHb + Mb represents oxygenated haemoglobin and oxygenated myoglobin, and DeoxyHb + Mb represents deoxygenated haemoglobin and deoxygenated myoglobin as determined by the NIRS device. Baseline TSI (PRE-TSI) was averaged from 2.5 to 12.5 min of the pre-IPC/SHAM rest period. The TSI during the IPC (IPC-TSI) and SHAM (SH-TSI) procedures was averaged during the final 5 s of each 5 min occlusion bout. An average 30 min stable period, ≥10 min after IPC/SHAM, was also calculated (POST-TSI). During the exercise testing, TSI was averaged over the final 60 s of the 20 W phase and every 30 ± 2 s throughout exercise and in the final 5 s of the TTE test. A single-exponential model, without time delay, with the fitting window commencing at $t = 0$ s, was used to calculate the kinetics of the change in deoxygenated haemoglobin and myoglobin from baseline during the whole trial as described in the following equation:

$$\mathrm{DeoxyHb} + \mathrm{Mb}\,(t) = \mathrm{DeoxyHb} + \mathrm{Mb\ baseline} + A\left(1 - e^{-(t/\mathrm{MRT})}\right), \quad (3)$$

where DeoxyHb + Mb(t) represents the absolute deoxygenated Hb + Mb at a given time t, DeoxyHb + Mb baseline represents the mean deoxygenated Hb + Mb over the final 60 s of baseline, and A and MRT represent the amplitude and mean response time of the whole DeoxyHb + Mb response, respectively.

2.12 | Statistical analysis

All statistical analysis was completed using SPSS (v.24, IBM Statistics, Armonk, New York, USA). Student's paired t-tests were used to compare and $\dot{V}_{O_2}$ and DeoxyHb + Mb kinetic parameters. Two-factor repeated-measures ANOVA, with time and condition (IPC vs. SHAM), were used to compare differences in TSI, mitochondrial function and RPE. Where significant effects were observed, least significant difference post-hoc paired t-tests were used to locate differences. Effect sizes (ESs) for t-test comparisons were calculated using Cohen's d statistic, interpreted using Hopkins' (2002) categorization criteria (0.2–0.59 = small; 0.6–1.19 = medium; >1.2 = large). All data are presented as the mean ± SD. Significance was accepted at $P \leq 0.05$.

3 | RESULTS

3.1 | Preliminary tests

The $\dot{V}_{O_2peak}$ and MAP were 52.7 ± 7.0 ml kg^{-1} min^{-1} and 318 ± 35 W, respectively. Vastus lateralis skinfold measurements did not differ between IPC and SHAM (8.4 ± 3.7 vs. 8.1 ± 3.0 mm, $P = 0.41$) and were within the photometric range of the NIRS device.

3.2 | Ischaemic preconditioning/SHAM protocol

Mean TSI during the occlusion phase was lower in IPC compared with SHAM (46 ± 5 vs. 69 ± 4%; $P < 0.0001$; ES = 3.3; Figure 1). Mean TSI did not differ between occlusion and reperfusion phases in SHAM ($P = 0.32$; ES = 0.38; Figure 1).

3.3 | Time-to-exhaustion test

There were no differences in TTE (249 ± 37 vs. 240 ± 32 s; $P = 0.62$; ES = 0.39; Figure 2), maximum HR (179 ± 8 vs. 180 ± 7 beats min^{-1}; $P = 0.86$; ES = 0.07), mean HR (170 ± 7 vs. 170 ± 7 beats min^{-1}; $P = 0.82$; ES = 0.09), RPE at 2 min (15 ± 2 vs. 15 ± 2; $P = 0.78$; ES = 0.10) or RPE at exhaustion (20 ± 0 vs. 20 ± 0; $P = 0.35$; ES = 0.35) between IPC and SHAM.

3.4 | Tissue O_2 saturation index and muscle DeoxyHb + Mb kinetics during exercise

The TSI was lower during the 5 s period before exhaustion compared with rest in IPC and SHAM (−11 ± 6 vs. −12 ± 5 %; $P < 0.0001$; ES ≥ 2.42); however, there was no time × condition interaction ($P = 0.41$; Figure 3). There was no difference in DeoxyHb + Mb MRT between IPC and SHAM (13 ± 4 vs. 10 ± 3 s; $P = 0.16$; ES = 0.68).

3.5 | Mitochondrial respiration

There was no time × condition interaction effect for mass-corrected mitochondrial OXPHOS and ETS capacity through protein complexes I–IV (CIP, $P = 0.50$; CI+IP, $P = 0.33$; CI+IE, $P = 0.11$; CIIE, $P = 0.08$; CIVE, $P = 0.43$; Table 1). There was a significant time × condition interaction ($P = 0.05$) for mass-corrected CIL, which was greater postexercise compared with baseline in SHAM ($P = 0.01$; ES = 1.20) but not in IPC ($P = 0.19$; ES = 0.51; Table 1). Citrate synthase activity did not differ between conditions or pre- and post-intervention in SHAM (15.7 ± 3.0 vs. 14.4 ± 2.5 mol h^{-1} kg^{-1}; $P = 0.18$) or IPC (16.1 ± 3.7 vs. 15.4 ± 2.4 mol h^{-1} kg^{-1}; $P = 0.48$). There were no differences in mass-corrected baseline CIL between SHAM and IPC; however, this difference might be trending towards significance ($P = 0.095$; ES = 0.68). There was no time × condition interaction effect for CS activity-corrected mitochondrial OXPHOS and ETS capacity through protein complexes I, II and IV (CIP, $P = 0.31$; CI+IP, $P = 0.22$; CI+IE, $P = 0.14$; CIIE, $P = 0.06$; CIVE, $P = 0.94$; Table 2). There was a significant time × condition interaction ($P = 0.01$) for CS activity-corrected CIL, which was greater postexercise compared with baseline in SHAM ($P = 0.003$; ES = 1.61) but not in IPC ($P = 0.16$; ES = 0.53. Table 2). There were no differences in baseline CS activity-corrected CIL between SHAM and IPC; however, these data might be trending towards significance ($P = 0.052$; ES = 0.85). An additional difference

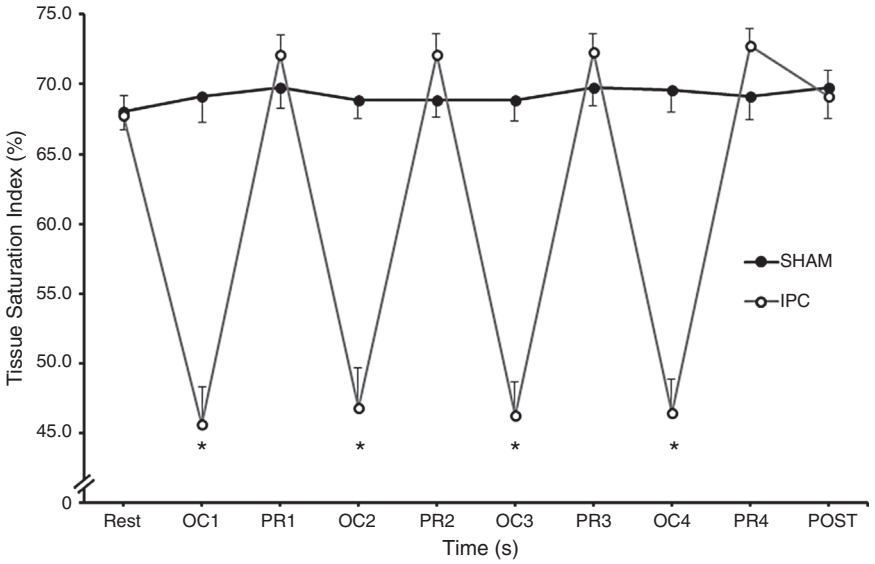

FIGURE 1 Tissue saturation index (TSI) throughout the ischaemic preconditioning (IPC) and sham-control (SHAM) procedures. The TSI was determined in the final 5 s of each period of occlusion (OC 1–4), 15 s after release of the occlusion cuff (PR 1–4) and over an average of the 5–30 min postinflation period (POST). $^{*}P \leq 0.05$ between IPC and SHAM. Data are means ± SD ($n = 8$)

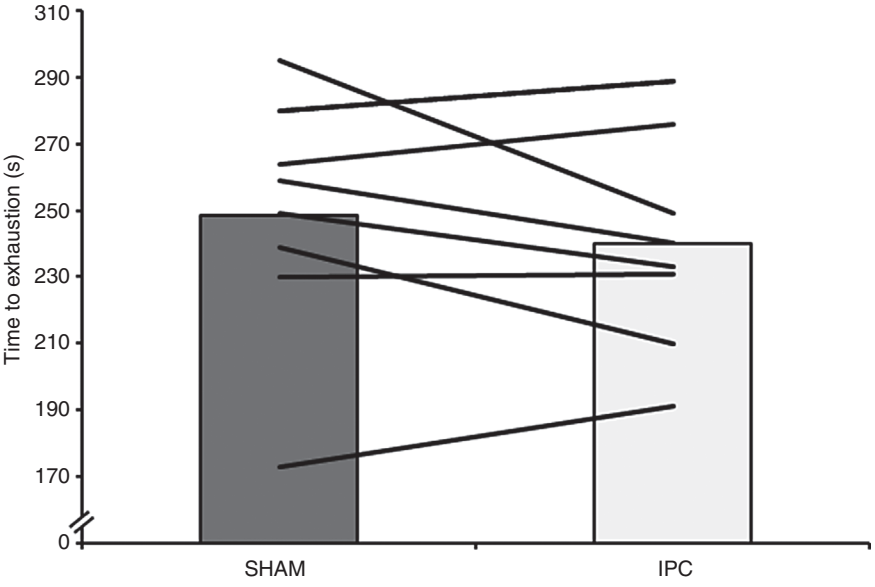

FIGURE 2 Time-to-exhaustion (TTE) after the sham-control (SHAM) and ischaemic preconditioning (IPC) trials. Bars represent the mean value. Lines represent individual responses ($n = 8$)

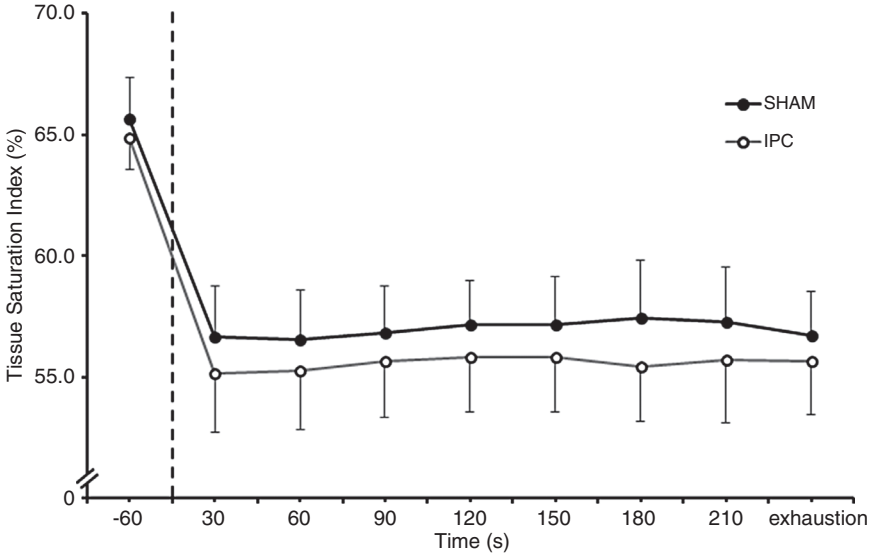

FIGURE 3 Tissue saturation index immediately before and throughout the time-to-exhaustion test in the sham-control (SHAM) and ischaemic preconditioning (IPC) trials. Data are the mean ± SD ($n = 8$)

TABLE 1 Parameters of mitochondrial respiration corrected to wet mass in SHAM and IPC conditions

Conditions		CIL	CIP	CI+IIP	CI+IIE	CIIE	CIVE
SHAM	Pre	1.9 ± 1.8	31.6 ± 6.4	52.5 ± 8.9	59.4 ± 11.0	22.6 ± 4.7	201.7 ± 31.7
	Post	4.5 ± 1.7*	35.5 ± 9.4	56.3 ± 12.2	66.8 ± 13.2	23.7 ± 4.8	188.2 ± 51.9
IPC	Pre	3.2 ± 1.6	40.6 ± 12.5	63.1 ± 11.6	73.2 ± 10.3	28.5 ± 8.6	199.5 ± 47.9
	Post	3.9 ± 2.2	40.4 ± 10.3	62.8 ± 13.9	72.2 ± 13.1	24.1 ± 5.0	213.9 ± 82.8

Abbreviations: CIL, leak respiration through mitochondrial protein complex (C) I of the electron transfer system; CIP and CI+IIP, ADP-stimulated respiration (P) through CI and CII of the electron transfer system, respectively; CI+IIE, CIIE and CIVE, maximal non-coupled electron transfer system capacity (E) of CI and CII, CII and CIV, respectively; Pre, before sham-control (SHAM) or ischaemic preconditioning (IPC); Post, immediately after time-to-exhaustion test. *Note*. Values are means ± SD, in picomoles per second per milligram. All data are $n = 8$.
*$P \leq 0.05$ compared with Pre.

TABLE 2 Parameters of mitochondrial respiration corrected to citrate synthase activity in SHAM and IPC conditions

Conditions		CIL	CIP	CI+IIP	CI+IIE	CIIE	CIVE
SHAM	Pre	0.10 ± 0.07	2.11 ± 0.46	3.53 ± 0.75	3.99 ± 0.90	1.53 ± 0.46	12.37 ± 3.85
	Post	0.29 ± 0.10*	2.42 ± 0.73	4.77 ± 0.78	4.48 ± 0.87	1.58 ± 0.30	12.87 ± 4.48
IPC	Pre	0.21 ± 0.10	2.75 ± 1.00	4.20 ± 0.93	4.98 ± 1.52	1.91 ± 0.66	14.13 ± 7.06
	Post	0.26 ± 0.15	2.61 ± 0.85	4.14 ± 0.89	4.80 ± 0.99	1.60 ± 0.38	14.76 ± 6.13

Abbreviations: CIL, leak respiration through mitochondrial protein complex (C) I of the electron transfer system; CIP and CI+IIP, ADP-stimulated respiration (P) through CI and CII of the electron transfer system, respectively; CI+IIE, CIIE and CIVE, maximal non-coupled electron transfer system capacity (E) of CI and CII, CII and CIV, respectively; Pre, before sham-control (SHAM) or ischaemic preconditioning (IPC); Post, immediately after time-to-exhaustion test. *Note*. Values are means ± SD, in picomoles per second per milligram. All data are $n = 8$.
*$P \leq 0.05$ compared with Pre.

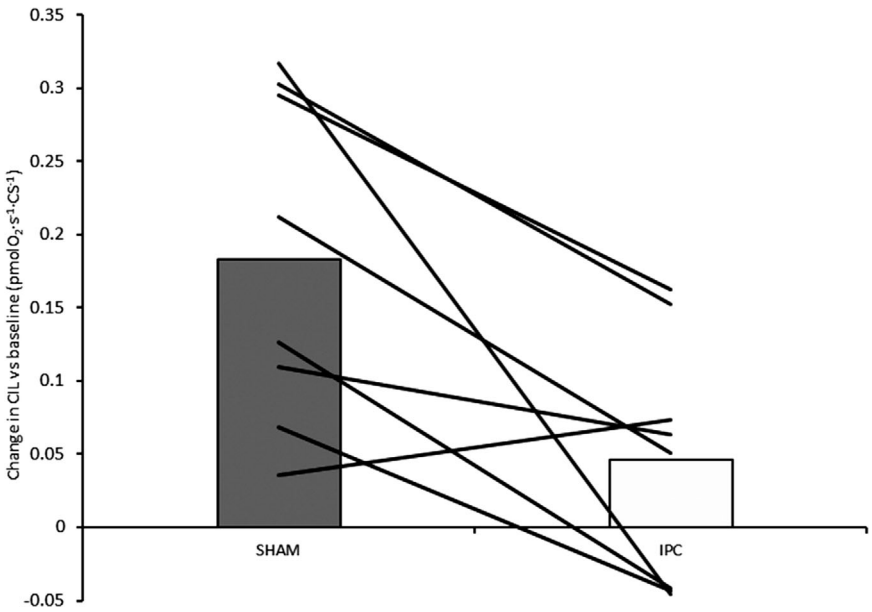

FIGURE 4 Change in citrate synthase activity (CS)-corrected leak respiration through mitochondrial protein complex I (CIL) after the time-to-exhaustion test compared with baseline in sham-control (SHAM) and ischaemic preconditioning (IPC) trials. *$P \leq 0.05$ between SHAM and IPC. Bars represent the mean value. Lines represent individual responses ($n = 8$)

in CS activity-corrected CIL was found between pre-exercise in the SHAM trial and postexercise in the IPC trial ($P = 0.050$; ES 0.86); however, the change in CIL between pre- and postexercise was greater in SHAM compared with IPC ($P = 0.01$; ES = 1.22; Figure 4). Further examination of the difference between SHAM and IPC at baseline revealed differences in mass-corrected CI+IIE ($P = 0.03$) and trends towards differences in CIP ($P = 0.058$) and CIP+IIP ($P = 0.06$; Table 1). Baseline differences between trials in CS activity-corrected CIP ($P = 0.03$) and CI+IIP ($P = 0.01$) were detected alongside trends in CI+IIE ($P = 0.10$; Table 2).

To account for these differences, mitochondrial respiration was also corrected to CI+IIP (Table 3), where a significant effect of time was found in CIL ($P = 0.02$). Post-hoc paired *t*-tests revealed no baseline CIL difference between SHAM and IPC at baseline ($P = 0.36$; ES = 0.35). The CIL was greater compared with baseline after exercise in SHAM ($P = 0.03$; ES = 0.94), but not in IPC ($P = 0.23$; ES = 0.47).

TABLE 3 Parameters of mitochondrial respiration corrected to ADP-stimulated respiration through mitochondrial protein complexes I and II

Conditions		CIL	CIP	CI+IIE	CIIE	CIVE
SHAM	Pre	0.04 ± 0.04	0.60 ± 0.07†	1.13 ± 0.05	0.43 ± 0.08	3.95 ± 0.96
	Post	0.08 ± 0.03*†	0.64 ± 0.13‡	1.19 ± 0.08	0.43 ± 0.06	3.37 ± 0.76
IPC	Pre	0.05 ± 0.03	0.53 ± 0.10	1.18 ± 0.16	0.45 ± 0.11	3.29 ± 1.20
	Post	0.06 ± 0.03	0.54 ± 0.13	1.16 ± 0.10	0.39 ± 0.09	3.45 ± 1.09

Abbreviations: CIL, leak respiration through mitochondrial protein complex (C) I of the electron transfer system; CIP and CI+IIP, ADP-stimulated respiration (P) through CI and CII of the electron transfer system, respectively; CI+IIE, CIIE and CIVE, maximal non-coupled electron transfer system capacity (E) of CI and CII, CII and CIV, respectively; Pre, before sham-control (SHAM) or ischaemic preconditioning (IPC); Post, immediately after time-to-exhaustion test. *Note*. Values are means ± SD, in picomoles per second per milligram. All data are $n = 8$.
*$P \leq 0.05$ compared with Pre.
†$P \leq 0.05$ compared with IPC Pre.
‡$P \leq 0.05$ compared with IPC Post.

TABLE 4 Citrate synthase activity corrected mitochondrial respiratory flux control ratios in SHAM and IPC conditions

Conditions		LCR	PCR	InvRCR	SCR	CIVres
SHAM	Pre	0.03 ± 0.02	0.53 ± 0.06	0.05 ± 0.04	0.60 ± 0.07	0.32 ± 0.15
	Post	0.07 ± 0.03*	0.51 ± 0.08	0.14 ± 0.06*	0.61 ± 0.12	0.31 ± 0.07
IPC	Pre	0.04 ± 0.02	0.55 ± 0.15	0.08 ± 0.04	0.64 ± 0.13	0.34 ± 0.10
	Post	0.05 ± 0.03	0.55 ± 0.10	0.10 ± 0.05	0.63 ± 0.09	0.31 ± 0.11

Abbreviations: LCR, leak control ratio, the quotient of leak respiration (CIL) over uncoupled electron transfer system capacity (E) of CI and CII (CI+IIE); PCR, phosphorylation control ratio, the quotient of ADP-stimulated respiration (P) through CI and CII of the electron transfer system (CI+IIP) over CI+IIE; InvRCR, inverse respiratory control ratio the quotient of CIL over CI+IIP; SCR, substrate control ratio, the quotient of CIP over CI+IIP at constant oxidative phosphorylation; CIVres, reserve capacity of mitochondrial protein complex IV, the quotient of CI+IIP over CIVE; Pre, before sham-control (SHAM) or ischaemic preconditioning (IPC); Post, immediately after time-to-exhaustion test. *Note*. Values are means ± SD, in picomoles per second per milligram. All data are $n = 8$.
*$P \leq 0.05$ compared with Pre.

Postexercise CIL in SHAM was also different to baseline CIL in IPC ($P = 0.05$; ES = 0.86). A trial effect for CI+IIP-corrected CIP was also found ($P = 0.024$); however, there was no difference in this variable in either set of conditions between time points in post-hoc paired *t*-tests ($P \geq 0.34$), with greater CIP found in SHAM at baseline and postexercise compared with baseline in IPC ($P = 0.05$ and $P = 0.04$, respectively).

Time × condition interactions for LCR ($P = 0.02$) and InvRCR ($P = 0.02$) were found, with both being greater postexercise compared with baseline in SHAM ($P = 0.004$; ES = 1.46 for LCR; $P = 0.006$; ES 1.37, for InvRCR) but not in IPC ($P = 0.26$; ES = 0.43 for LCR; $P = 0.30$; ES = 0.40 for InvRCR; Table 4). Furthermore, a difference was found between InvRCR at baseline in IPC and postexercise in SHAM ($P = 0.04$; ES = 0.92). Neither LCR ($P = 0.10$; ES = 0.66) or InvRCR ($P = 0.14$; ES = 0.60) differed at baseline between SHAM and IPC. No interactions were observed in any of the other FCRs (PCR, $P = 0.92$; SCR, $P = 0.70$; CIVres, $P = 0.68$; Table 4).

3.6 | Pulmonary oxygen uptake kinetics

The $\dot{V}_{O_2}$ MRT was faster in IPC compared with SHAM when the fitting window was applied to both the entire $\dot{V}_{O_2}$ response ($P = 0.003$; ES = 1.57) and the 210 s isotime ($P = 0.05$; ES = 0.87; Figure 5). There was a smaller slow component in IPC compared with SHAM when the fitting window was applied to both the entire $\dot{V}_{O_2}$ response ($P = 0.03$; ES = 0.97) and the 210 s isotime ($P = 0.01$; ES = 1.28). There were no differences in any other parameters of $\dot{V}_{O_2}$ kinetics ($P > 0.05$; Table 5).

4 | DISCUSSION

The main findings of the present study are that administration of IPC before severe-intensity exercise: (1) appeared to prevent an increase in leak respiration, proportional to maximal ETS and ADP-stimulated respiration, that was evoked by severe-intensity exercise in SHAM; (2) expedited pulmonary $\dot{V}_{O_2}$ kinetics during exercise; but (3) did not improve exercise performance or alter TSI during exercise.

In the present study, severe-intensity exercise increased CIL. This arises either as a result of re-entry of protons into the mitochondrial matrix without entering through the complex V ATP synthase channel to produce ATP or through proton leak/slip during electron transfer by mitochondrial CI and CII. The result of this proton leak is reduced efficiency of ATP resynthesis. Increased leak respiration has previously been demonstrated after high-intensity, sprint-interval and continuous

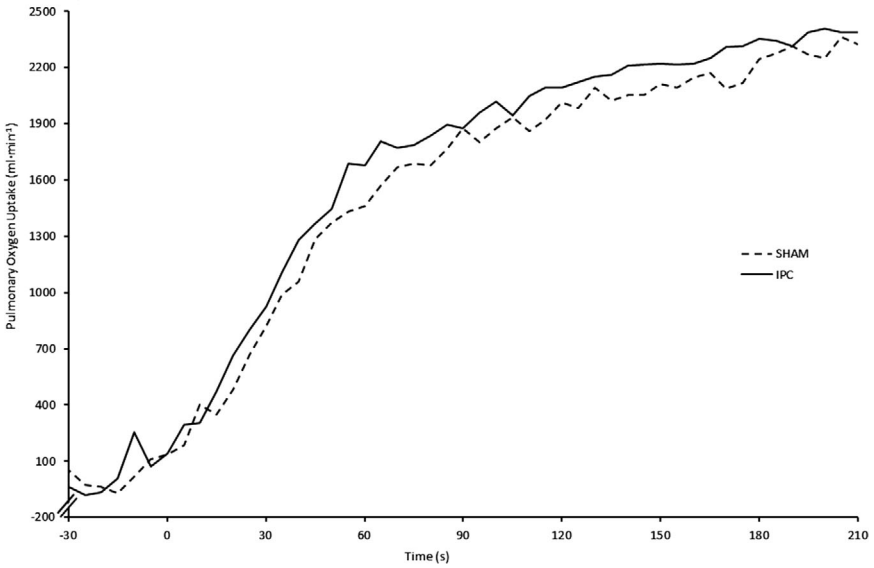

FIGURE 5 Pulmonary oxygen uptake response during the final 30 s of baseline measurements and throughout the time-to-exhaustion test in sham-control (SHAM) and ischaemic preconditioning (IPC) trials. Lines are the group mean responses ($n = 8$) corrected to baseline oxygen uptake

TABLE 5 Oxygen uptake kinetics during the time-to-exhaustion test in SHAM and IPC conditions, when the fitting window was applied to the entire oxygen uptake response and to a fixed isotime of 210 s

	Entire $\dot{V}_{O_2}$ response			Isotime (210 s)		
Parameter	SHAM	IPC	*P*-value	SHAM	IPC	*P*-value
Baseline $\dot{V}_{O_2}$, ml min^{-1}	1,492 ± 315	1,372 ± 242	0.227	1,534 ± 314	1,385 ± 258	0.180
$\dot{V}_{O_2}$ at 120 s, ml min^{-1}	3,510 ± 287	3,561 ± 305	0.496	3,499 ± 308	3,543 ± 325	0.609
End-exercise $\dot{V}_{O_2}$, ml min^{-1}	3,885 ± 316	3,831 ± 366	0.435	3,869 ± 356	3,856 ± 420	0.888
Mean response time, s	63.7 ± 14.5	51.3 ± 15.5	0.003	61.1 ± 14.7	52.4 ± 17.3	0.050
Amplitude, ml min^{-1}	2,426 ± 396	2,478 ± 247	0.524	2,332 ± 439	2,453 ± 262	0.254
$\Delta\dot{V}_{O_2}$ 120 s–end, ml min^{-1}	377 ± 188	270 ± 105	0.028	370 ± 101	313 ± 130	0.011
End-exercise $\dot{V}_{O_2}$, $\%\dot{V}_{O_2peak}$	94 ± 5	93 ± 5	0.428	93 ± 6	93 ± 5	0.829

Abbreviations: SHAM, sham-control trial; IPC, ischaemic preconditioning trial; $\Delta\dot{V}_{O_2}$ 120 s–end, change in oxygen uptake ($\dot{V}_{O_2}$) from 120 s to the end of the time-to-exhaustion test; $\dot{V}_{O_2peak}$, peak $\dot{V}_{O_2}$ in the final minute of the incremental ramp test to exhaustion. *Note*. Values are means ± SD. All data are $n = 8$ except for the isotime data, for which $n = 7$ because data from one participant were omitted to avoid over-constraining the fitting window, given that the slowest time to exhaustion was considerably lower than the group mean time to exhaustion (173 vs. 253 s).

moderate-intensity exercise (Trewin et al., 2018). However, the exercise-induced increase in CIL that occurred in the SHAM conditions did not occur when exercise was preceded by IPC. Furthermore, the increases in internal FCRs (LCR and InvRCR) after SHAM are indicative of increased leak relative to ADP-stimulated respiration and maximal ETS capacity. These increases did not occur after IPC. To our knowledge, this is the first study to demonstrate that IPC might preserve human skeletal muscle mitochondrial coupling efficiency, via reduced leak respiration relative to ADP-stimulated and maximal mitochondrial respiration, during exercise. These observations are broadly consistent with clinical research reporting attenuated leak respiration after IPC in mitochondria isolated from cardiac muscle (Nadtochiy et al., 2006; Quarrie et al., 2011). The present findings suggest that IPC might also maintain mitochondrial coupling efficiency postexercise in situ.

It is well established that, during fatiguing exercise, such as the TTE test undertaken in the present study, the muscle environment becomes more acidic and contractility is impaired. There is evidence that some of the intramyocyte metabolic perturbations that accompany fatiguing muscle contractions, such as increased oxidant production and cytosolic [Ca^{2+}] and low ATP content (Allen et al., 2008), can provoke the opening of mitochondrial permeability transition pores. In turn, opening of the mitochondrial permeability transition pores results in the release of protons and pro-apoptotic factors from the mitochondrial matrix and, subsequently, a decrease in the mitochondrial membrane potential and increase in leak respiration ensues (Fernström et al., 2004). Preconditioning exercise, involving 75 min of cycling at 70% of maximal $\dot{V}_{O_2}$, has been demonstrated to protect against mitochondrial swelling and rupture caused by successive additions of Ca^{2+}, as seen in fatiguing muscle in vitro

(Fernström et al., 2004). This exercise stimulus prepares the cells to tolerate Ca^{2+} and intramuscular acidosis better. The ischaemic stimulus imposed by IPC might evoke some similar protective mechanisms against the opening of mitochondrial permeability transition pores, resulting in the maintenance of leak respiration. Indeed, recent evidence suggests that IPC facilitates the removal of Ca^{2+} during ischaemia while hydroxyl radicals remain in the mitochondrial matrix (Dolowy, 2019). The resultant increased buffering capacity afforded by calcium hydroxide might then facilitate an improved ability to tolerate subsequent acidosis, via a blunted increase in exercise-induced leak respiration from the mitochondrial matrix. However, it is important to recognize that additional mechanical and metabolic stimuli are induced by exercise compared with an IPC stimulus and, as such, further research is required to resolve the mechanisms underpinning the attenuated increase in exercise-induced leak respiration after IPC administration.

Ischaemic preconditioning reduced the change in $\dot{V}_{O_2}$ between 120 s and the end of exercise compared with SHAM, which is suggestive of a lower $\dot{V}_{O_2}$ slow component. Furthermore, faster overall $\dot{V}_{O_2}$ kinetics were observed after IPC, as reflected in the lower MRT compared with SHAM. However, it should be acknowledged that a limitation of the $\dot{V}_{O_2}$ kinetics analysis conducted in the present study was that precise modelling of phase II $\dot{V}_{O_2}$ kinetics and determination of the $\dot{V}_{O_2}$ slow component onset and magnitude were not possible owing to the single severe-intensity exercise transition for each of the experimental conditions. It is well established that the $\dot{V}_{O_2}$ slow component originates principally in skeletal muscle (Poole et al., 1991). This has been linked to increased fibre recruitment, particularly of type II fibres, which have a higher ATP cost of force production and are, therefore, less efficient than type I muscle fibres (Poole & Jones, 2012). However, it is possible to observe a $\dot{V}_{O_2}$ slow component in the absence of progressive recruitment of muscle fibres (Zoladz et al., 2008), where altered mitochondrial function might be a contributory factor. In the present study, increased CIL with no change in ADP-coupled respiration or ETS capacity, supported by increased InvRCR and LCR in the SHAM conditions, implies that the reduciton in mitochondrial coupling efficiency with severe-intensity exercise could contribute to the development of the $\dot{V}_{O_2}$ slow component. Moreover, the observation that increases in CIL, LCR and InvRCR after severe-intensity exercise in SHAM were not apparent after IPC, concomitant with a reduced $\dot{V}_{O_2}$ slow component, corroborates this interpretation.

The faster pulmonary $\dot{V}_{O_2}$ kinetics with no change in DeoxyHb + Mb kinetics, which provides an estimate of muscle O_2 extraction (Koga et al., 2012), implies that the faster $\dot{V}_{O_2}$ MRT after IPC was evoked by a proportional increase in muscle O_2 delivery kinetics that facilitated greater muscle O_2 utilization. The mechanism by which IPC might influence O_2 delivery is postulated to result from a reperfusion-induced humoral signalling cascade (Wang et al., 2004). Indeed, previous research demonstrates enhanced blood flow to skeletal muscle, preservation of endothelial function, enhanced functional sympatholysis and, ultimately, a greater O_2 delivery after IPC (T. G. Bailey, Birk et al., 2012; Beaven et al., 2012; Horiuchi et al., 2015; Wang et al., 2004).

Despite the maintenance of mitochondrial respiratory function postexercise and faster overall $\dot{V}_{O_2}$ kinetics, IPC had no effect on exercise performance. It is possible to dissociate alterations in $\dot{V}_{O_2}$ kinetics and mitochondrial respiration from exercise performance. For example, in research examining the optimization of prior exercise priming strategies it was observed that interventions resulting in the biggest enhancements of $\dot{V}_{O_2}$ kinetics (lower MRT and $\dot{V}_{O_2}$ slow component) can compromise exercise performance (S. J. Bailey et al., 2009). Moreover, improvements in mitochondrial function as a result of exercise training can be observed that do not always translate into improvements in performance (Granata et al., 2016). There is also a suggestion that a phenotype for responsiveness to IPC might exist, with certain prerequisites for success of the intervention, for example, the responder versus non-responder hypothesis proposed by Incognito et al. (2016). In the present study, individual responses were variable, with 50% of the participants performing better after IPC compared with SHAM. However, research exploring what constitutes this phenotype in the literature is limited, and further research is required to determine the optimization and context of strategies, such as IPC, that manipulate $\dot{V}_{O_2}$ kinetics, mitochondrial respiration and exercise performance.

The present study is not without limitations. The SUIT protocol used in this study aimed to evaluate several parameters of mitochondrial function. However, given the findings of preserved mitochondrial coupling efficiency, largely driven by greater leak respiration after exercise in SHAM but not IPC, a SUIT protocol providing greater insight into leak respiration would have improved confidence in concluding that IPC attenuates exercise-induced increases in mitochondrial leak respiration. Therefore, future research might benefit from the use of oligomycin to inhibit mitochondrial CIV ATP synthase (Devenish et al., 2000) to provide further insight into the effect of IPC on exercise-induced alterations in leak respiration. A further consideration is the inherent complexity of comparing mitochondrial respiratory parameters pre- and postexercise and how to account for potential inter-biopsy differences in mitochondrial content. There were no differences in CS activity across time or between conditions in the present study, consistent with previous studies using a similar exercise intensity and duration (Layec et al., 2018; Lewis et al., 2021), suggesting that this mitochondrial content normalization procedure is appropriate for these data given the exercise test administered in the present study. Furthermore, any exercise-induced changes in wet mass or CS activity, used to normalize the mitochondrial respiratory data, would be expected to impact all respiratory variables, in both conditions, which would be expected to result in no interaction effect and no changes in internal FCR. Therefore, our observations are unlikely to be confounded by the normalization procedures adopted in the present study.

Trends towards a difference in baseline CIL between conditions in the CS activity-corrected normalization approach ($P = 0.052$) and, to lesser degree, in the mass-corrected normalization approach ($P = 0.095$) allowed further examination of baseline high-resolution respirometry variables between conditions. Specifically, Student's paired *t*-tests revealed lower CIP and CI+IIP in SHAM compared

with IPC. Taken together, the data suggest that SHAM baseline high-resolution respirometry might be lower owing to the inherent variability of examining different muscle biopsies using this technique. Furthermore, variability of CS activity in different samples obtained from the same muscle biopsy might also be a contributory factor. The variability of resting high-resolution respirometry data in this study is within that previously reported in the literature (Cardinale et al., 2018; Kuang et al., 2022); nevertheless, this remains an important consideration, and caution should be exercised when interpreting the results relating to CIL. Examination of the relative contributions of CIL to P and E, demonstrated in InvRCR and LCR, therefore provides a more appropriate representation of the potential for IPC to maintain mitochondrial coupling efficiency. Our findings demonstrateed increased leak respiration relative to P (InvRCR) and E (LCR) from the same biopsy in SHAM after exercise, but not IPC, where baselines did not differ ($P = 0.14$ and $P = 0.10$, respectively). Furthermore, the CI+IIP-corrected data used an internal measure of mitochondrial content, in the same analysis of high-resolution respirometry, which might then account for the lower baseline respiration in SHAM compared with IPC. The CI+IIP is demonstrated to correlate strongly with mitochondrial content, determined by fractional area ($r = 0.81$, $P < 0.001$; Larsen et al., 2012) and was found to be significantly lower at baseline in SHAM compared with IPC. Correcting to CI+IIP as a marker of mitochondrial content might then be useful to adjust for baseline differences and to reduce the influence of variability in CS activity in different samples from the same muscle biopsy (Kuang et al., 2022). These data collectively corroborate the finding of greater CIL after exercise in SHAM, but not IPC, evidenced in the more established normalizations to wet mass and CS activity, again with no difference in the baselines ($P = 0.36$). Importantly, the findings of an attenuated exercise-induced increase leak respiration with pre-exercise IPC in this study are manifest irrespective of whether CIL is corrected to wet mass or CS activity and when inferred from internally normalized FCRs (InvRCR and LCR).

In conclusion, IPC expedited overall $\dot{V}_{O_2}$ kinetics and appeared to prevent an increase in leak respiration, proportional to maximal ETS and ADP-stimulated respiration, that was evoked by severe-intensity exercise in SHAM. However, despite these physiological improvements, the performance of severe-intensity exercise was not enhanced after IPC. Although IPC improved aspects of oxidative metabolism that could potentially be ergogenic in certain exercise settings, the findings of the present study do not support IPC as a pre-exercise strategy to improve the performance of severe-intensity exercise in recreationally active participants.

AUTHOR CONTRIBUTIONS

Donald L. Peden, Stephen J. Bailey and Richard A. Ferguson contributed to the conception and design of the experiment. All authors contributed to the acquisition, analysis and interpretation of data and drafting or critical revision of the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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COMPETING INTERESTS

None declared.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this research is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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