Cocking, S, Wilson, MG, Nichols, D, Cable, NT, Green, DJ, Thijssen, DHJ and Jones, H

Is There an Optimal Ischaemic Preconditioning Dose to Improve Cycling Performance?

Is There an Optimal Ischaemic Preconditioning Dose to Improve Cycling Performance?

Scott Cocking [1, 2],

Mathew G. Wilson [1,2]

David Nichols [1, 2]

N. Timothy Cable [2, 5]

Daniel J. Green [2],[3]

Dick H. J. Thijssen [2, 4]

Helen Jones [2]

[1] Athlete Health and Performance Research Centre, *Aspetar Orthopaedic and Sports Medicine Hospital*, Doha, Qatar.

[2] Research Institute for Sport and Exercise Science, *Liverpool John Moores University*, UK

[3]School of Sports Science, Exercise and Health, The University of Western Australia, Crawley, Perth, Western Australia, Australia

[4]Radboud Institute of Health Sciences, Department of Physiology, *Radboud University Medical Center*, the Netherlands

[5] Department of Sport Science, Aspire Academy, Doha, Qatar

SHORT TITLE: Optimal IPC dose for cycling time trial

WORD COUNT (MANUSCRIPT): **3631**
Abstract word count: 243
Number of References: 32
Tables: 4

Author for correspondence:

Prof Helen Jones, Research Institute of Sports and Exercise Science, Liverpool John Moores University, Tom Reilly Building, Byrom Street, Liverpool, L3 3AF
h.jones1@ljmu.ac.uk
TEL: +441519046270

DISCLOSURE: No conflicts of interest

Abstract

INTRODUCTION: Ischaemic preconditioning (IPC) may enhance endurance performance. No previous study has directly compared distinct IPC protocols for optimal benefit. The aim of this study was to determine whether a specific IPC protocol (i.e. number of cycles, amount of muscle tissue, and local vs remote occlusion) elicits greater performance outcome.

METHODS: Twelve cyclists performed five different IPC protocols 30-min prior to a blinded 375 kJ cycling time trial (TT) in a laboratory. Responses to traditional IPC (4x5-min legs) were compared to: *i.* 8x5-min legs and SHAM ("dose-cycles"), *ii*. 4x5-min unilateral legs ("dose-tissue"), and *iii*. 4x5-min arms ("remote"). RPE and blood lactate were recorded at each 25% TT completion. Power (watts), heart rate (bpm), and $\dot{V}O_2$ (ml.kg.min^{-1}) were measured continuously throughout TT's. Magnitude based inference statistics were employed to compare variable differences to the minimal practically important difference.

RESULTS: Traditional IPC was associated with a 17 (0, 34) secs faster TT time compared to SHAM. Applying more "dose-cycles" (8x5-min) had no impact on performance. Traditional IPC was associated with "likely trivial" higher blood lactate and "possibly beneficial" lower $\dot{V}O_2$ responses vs. SHAM. Unilateral IPC was associated with 18 (-11, 48) secs slower performance compared to

bilateral ("dose-tissue"). TT times following remote and local IPC were not different [0 (-16, 16) secs].

CONCLUSION: The traditional 4x5-min (local or remote) IPC stimulus resulted in the fastest TT time compared to SHAM, there was no benefit of applying a greater number of cycles or employing unilateral IPC.

Key words: Exercise, Occlusion, Ischaemia, Time Trial, Endurance

Introduction

Ischaemic preconditioning (IPC) refers to the phenomenon whereby 3-4 brief periods of ischaemia, followed by tissue reperfusion, confers subsequent tissue protection against ischaemic insult [1]. IPC can be applied remotely by placing a blood pressure cuff around a limb and inflating to supra-systolic pressure. Studies have generally employed remote IPC in clinical populations relating to cardio-protection, but there is accumulating evidence that remote IPC can impact on other organs (e.g. skeletal muscle), and vascular beds to facilitate increased blood flow [2,3]. These finding have resulted in the application of IPC to determine its efficacy as a potential pre-exercise priming strategy.

The first study to investigate IPC in a human exercise model demonstrated a 3% improvement in maximal oxygen uptake ($\dot{V}O_2$)

following a 3x5-min bilateral leg cuff inflation (220 mmHg) protocol [4]. A "traditional" IPC protocol consists of 3x5- or 4x5-min bouts of occlusion. More recently, studies have separately employed alternative IPC protocols (altering the number of IPC cycles, tissue occlusion area, and cuff location), with the aim of observing greater performance and clinical outcomes. There are now (pre)clinical studies providing evidence for a "dose"-dependency, where repeated daily IPC improves (cerebro)vascular function and clinical outcomes [5,6]. Nonetheless, a potential 'hyper-conditioning' effect from excessive cycles of IPC cannot be excluded [7]. Corroborating the "dose"-hypothesis, recent work suggests that bilateral, but not unilateral cuff inflation leads to improved exercise performance [8]. Finally, most studies to date have opted for cuff positioning directly on the exercising limb [9], but cuff placement on remote, non-exercising limbs has also been performed [10] to examine a systemic effect. In line with clinical observations in the protection of organs against ischaemic injury, local or remote application of IPC may induce comparable benefits [2,11].

Recently, a systematic review and meta-analysis reported a small beneficial effect of IPC on exercise performance, with the largest effect observed in aerobic-based tasks [12]. Despite the effect sizes being small, the potential benefits of IPC may translate to meaningful differences in competitive (time trial-based) events.

Interestingly, no study has directly compared the capacity of distinct IPC protocols with the aim of electing greater performance improvement. Therefore, the aim of this study was to examine whether the (i) number IPC cycles (i.e. "dose-cycles"), (ii) the amount of muscle mass occluded ("dose-tissue"), and (iii) the application of IPC to either local or remote limbs ("remote") offers greater improvements to endurance cycling performance.

Methods

Participants

Twelve trained cyclists (mean±SD: age, 36±7 years; body mass, 78±4 kg; height, 179±6 cm; $\dot{V}O_{2max}$, 59±4 $ml.kg^{-1}.min^{-1}$) were recruited. Participants were undertaking regular weekly training sessions (5±3 sessions) and mean weekly training volume was 8±4 hours. The mean training experience was 9±8 years. Following verbal and written explanation of procedures, all participants provided written informed consent. Physical Activity Readiness Questionnaires were administered to ensure no participant had any health implications that would prevent participation. All individuals refrained from exercise and alcohol consumption 24 hours, and consumption of caffeine at least 6 hours, respectively prior to all laboratory visits. The study was approved by the local Ethics Committee.

Research Design

The study was divided into three comparisons as illustrated in figure 1. All participants completed a maximal graded cycling test and at least two familiarization TT. Prior to commencement of the five experimental cycling TT's, an IPC protocol was administered. A traditional (4x5-min) IPC protocol was compared firstly to SHAM, and a larger (8x5-min) IPC protocol for the "dose-cycles" comparison. Whilst it was compared to a unilateral (4x5-min) IPC protocol for the "dose-tissue" comparison. Finally, to assess the importance of cuff placement, a 4x5-min bilateral IPC protocol was applied to the non-exercising upper limb for the "remote" comparison.

Experimental Protocol

In a randomized, counterbalanced, crossover study, participants reported to the laboratory at the same time of day on five separate occasions, at least 4 days apart, receiving a different pre-exercise IPC protocol during each visit. Following each IPC protocol, a 20-minute rest period, and a standardized warm up was performed before the completion of a 375 kilojoule (kJ) cycling time trial (TT). The TT was intended to simulate the demands of a 16.1 km TT. During each TT, heart rate and oxygen uptake ($\dot{V}O_2$) was measured continuously, whilst blood lactate and rate of perceived of exertion

(RPE) was recorded at every 25% completed of the TT kilojoule target.

Measurements

Assessment of maximal oxygen uptake ($\dot{V}O_{2max}$). At least 7 days prior to the first familiarisation trial, participants performed a continuous incremental step test on an electromagnetically braked cycle ergometer (SRM, Julich, Germany) to determine lactate threshold and $\dot{V}O_{2max}$. The incremental protocol consisted of 3-minute cycling stages, commencing at 95 watts (W) and increasing 35W until volitional exhaustion occurred. Blood lactate concentration was obtained via finger prick capillary sampling using a safety lancet (BD Microtainer® Contact-Activated Lancet) after administration of a disposable sterile isoprophyl alcohol swab (China MEHECO Co., Ltd.). Blood was collected into a sodium-heparinized blood gas capillary tube (Marienfeld Superior, Germany) and immediately analysed in duplicate (ABL90 FLEX, Radiometer Medical ApS, Denmark) during the last 30 seconds of each incremental stage. Throughout the incremental cycling test, breath-by-breath expired gases were monitored for oxygen consumption, ventilation and respiratory exchange ratio (RER) (MasterScreen™ CPX, Carefusion, Germany) and the highest 30-second average was taken from 3 consecutive 10-second bins to subsequently determine $\dot{V}O_{2max}$. Heart rate (HR) was also monitored continuously

(Polar H1, Kempele, Finland). W_{max} was calculated from the last completed workload, plus the fraction of time spent in the final non-completed stage multiplied by the work rate increment [13].

Familiarisation. At least 2 familiarisation trials were undertaken prior to the first experimental TT to ensure performance was reliable. Data from familiarisation sessions revealed a mean coefficient of variation (CoV) of 1.06% which was deemed to be acceptable for the purpose of this TT study.

IPC protocols. For the IPC and SHAM trials, 13.5 cm wide cuffs were used. Participants lay in the supine position and cuff inflation pressure was set at a standardized pressure (220mmHg) in all IPC conditions with the aim of preventing arterial inflow [14] and 20mmHg in SHAM (i.e. cuffs were placed but only inflated to 20mmHg) with the use of an automatic rapid cuff inflator (Hokanson, Washington, USA). Subsequently, cuffs were deflated for 5 minutes, allowing reperfusion. This process was repeated four times in all protocols except for the "dose-cycles" protocol where 8 cycles were used (Figure 1). For IPC on the leg, the cuff was placed (unilaterally or bilaterally) on the most proximal portions of the upper thigh (distal to the inguinal fold). For remote IPC, cuffs were placed on the most proximal portions of the upper arms. Each participant gave a "perceived discomfort" rating at four time points (every 25%) throughout the IPC or SHAM protocols. The discomfort rating was

established using a Numerical Rating Scale (NRS) ranging from 0 (no discomfort) to 10 (maximum discomfort) and are included for descriptive purpose (Table 4) [15].

375 kJ TT. After 20 minutes of rest following cessation of IPC/SHAM, a capillary blood lactate sample was obtained from the finger and analysed for resting lactate levels (ABL90 FLEX, Radiometer Medical ApS, Denmark). Participants then completed a standardized warm up on an electromagnetically braked cycle ergometer (SRM, Julich, Germany). The warm up lasted approximately 10 minutes (5-min at 100W, 2-min at 150W, [15-secs at W_{max}, 30-secs at 150W, repeat x3], 45-secs at 150W). Once the flywheel had completely stopped turning, the SRM clock was reset to zero and a 375 kJ TT was performed (exactly 35 minutes after completion of IPC in all trials). Participants were instructed to produce a maximum effort throughout TT's, but were blinded to power output, elapsed time and HR. Breath-by-breath expired gases and HR were measured continuously, while RPE and blood lactate measurements were acquired at 25%, 50%, 75% and 100% time points (all described previously). Participants were notified once they had completed each quarter of the TT and when they had 30 kJ of work remaining. No encouragement or feedback was given throughout any trial.

Statistical Analysis

The primary outcome variable was TT time and was analyzed using a repeated measures general linear modelling for "dose-cycles" (3 levels: SHAM, 4x5-min, 8x5-min) and paired t-tests for "dose-tissue" (2 levels: unilateral, bilateral) and 'remote' (2 levels: local, remote). For TT measures, $\dot{V}O_2$, power, lactate, HR, and RPE were analyzed using repeated measures general linear modelling. The least significant method was employed for pairwise comparisons [16]. Using a magnitude based inferences framework, the mean effect of each TT comparison for each variable was presented with the uncertainty of the estimates presented as 90% confidence intervals (appropriate SI units used for a given variable). The mean difference between each comparison were evaluated for their practical significance by pre-specifying the smallest worthwhile change (SWC) [17]. For TT time and power output, the SWC was calculated using 0.3 x coefficient of variation from the familiarization trials, equating to 4.5 seconds and 1 watt, respectively [18]. The noise to signal ratio was determined by calculating the typical error (SD of between-trial differences divided by $\sqrt{2}$). The typical error for time and power was 18 seconds and 4 watts, respectively. For blood lactate and $\dot{V}O_2$ the SWC was calculated using the standardized mean difference of 0.2 between subject standard deviations (SD) as they were not measured during the familiarisation trials [19]. The SHAM values were used for this purpose. The mean difference

between each comparison, together with its uncertainty, the probability (percent chances) that the true population effect was beneficial (>SWC), harmful (>SWC with opposite sign), or trivial (within ± SWC) was calculated [18]. Using mechanistic inferences, qualitative probabilistic terms for benefit were assigned to each effect using the following scale; <0.5%, most unlikely or almost certainly not; 0.5 to 5%, very unlikely; 5 to 25%, unlikely or probably not; 25 to 75%, possibly; 75 to 95%, likely or probably; 95 to 99.5%, very likely; >99.5%, most likely or almost certainly [18]. An unclear effect is possibly beneficial (>25%) with an unacceptable risk of harm (>0.5%) and an odds ratio for benefit:harm of <66 interpreted from current recommendations; all other effects are clear. Data that were lower than the typical error (noise > signal) for TT performance were interpreted as "unclear" and reported with the confidence limits within the text and in figure 2.

Results

Dose-cycles

TT time: TT time was 17 secs (90% CI: 0, 34 secs; *P*=0.097) faster following the traditional IPC protocol compared to SHAM. The mean change is lower than the noise so is interpreted as "unclear" with the following confidence limits 89% chance beneficial, 9% chance trivial and 2% chance harmful (Figure 2b). Increasing the "dose" by applying more cycles (8x5-min) did not result in a faster

TT time compared to traditional IPC (4x5-min) [13 secs (-19, 44 secs); P=0.49, (beneficial 67%, trivial 15%, harmful 18%)] Figure 2]. The effect between IPC with 8x5-min cycles and SHAM on exercise performance was interpreted as "unclear" (beneficial 50%, trivial 19%, harmful 31%).

$\dot{V}O_2$: $\dot{V}O_2$ was 0.99 ml.kg.min^{-1} (-1.7, -0.3 ml.kg.min^{-1}) lower following traditional IPC compared to SHAM, interpreted as "possibly beneficial" (beneficial 59%, trivial 41%, harmful 0%; P=0.03). A "likely trivial" difference was evident between traditional IPC and the 8x5-min protocol [0.51 ml.kg.min^{-1}(-1.2, 0.2 ml.kg.min^{-1}); (beneficial 17%, trivial 83%, harmful 0%) P=0.25].

Lactate: Blood lactate increased throughout TT performance, with highest values observed during the 4th quarter (Table 1). Traditional IPC was associated with a higher mean TT blood lactate compared to SHAM [0.73 mmol.L^{-1} (0.1, 1.5 mmol.L^{-1}); P=0.06, "possibly trivial" (beneficial 42%, trivial 58%, harmful 0%)] and to the 8x5-min protocol [0.9 mmol.L^{-1} (0.4, 1.9 mmol.L^{-1}); P=0.006, "possibly beneficial" (beneficial 73%, trivial 27%, harmful 0%)].

Power / HR / RPE: HR and RPE increased significantly across time (P<0.05), whilst power was highest during the 1st quarter. No further differences were evident for power, HR, or RPE between traditional, SHAM and 8x5-min (all P>0.05; Table 1).

Dose-tissue

TT Time: Traditional bilateral IPC resulted in an 18 secs (-11, 48 secs, P=0.29; Figure 2) faster TT performance than unilateral IPC. Nevertheless, this change was interpreted as "unclear" (beneficial 78%, trivial 12%, harmful 10%).

$\dot{V}O_2$: The lower resultant $\dot{V}O_2$ following traditional IPC compared to unilateral IPC [0.8 ml.kg.min^{-1}; (-2, 0.4 ml.kg.min^{-1}); (beneficial 45%, trivial 54%, harmful 1%) P=0.26)] was interpreted as "possibly trivial". The time-dependent effect (Table 2), was not different between the 2 trials.

Lactate: Blood lactate increased throughout TT performance, with highest values during 4th quarter (Table 2). The mean blood lactate difference of 0.05 mmol.L^{-1} (-1.3, 1.4 mmol.L^{-1}); (beneficial 11%, trivial 81%, harmful 9%; P=0.95) between protocols was interpreted as "unclear".

Power / HR / RPE: HR and RPE increased significantly across time, whilst power was highest during the 1st quarter (Table 2) .No further differences were evident for power, HR, or RPE (Table 2).

Remote

TT time: The comparison of traditional IPC and remote IPC resulted in a negligible difference in mean TT time [0 secs (-16, 16 secs;

P=1.0, Figure 2a)]; interpreted as an "unclear" (beneficial 50%, trivial 0, harmful 50%).

$\dot{V}O_2$: $\dot{V}O_2$ was 1.1 ml.kg.min^{-1} (-1.9, -0.2 ml.kg.min^{-1}; (beneficial 71%, trivial 29%, harmful 0%) P=0.04) lower following the traditional protocol compared to remote IPC, interpreted as a "possibly beneficial" reduction.

Lactate: Blood lactate increased throughout both TT performances, with highest values observed during 4th quarter (Table 3). A mean blood lactate difference of 0.2 mmol.L^{-1} occurred (-1.2, 1.6 mmol.L^{-1}; P=0.8) between both protocols, interpreted as an "unclear" difference (beneficial 18%, trivial 74%, harmful 8%).

Power / HR / RPE: HR and RPE increased significantly across time, whilst power was highest during the 1st quarter. No further differences were evident for power, HR, or RPE between traditional and remote IPC (Table 3).

Discussion

The aim of this study was to determine the impact of different IPC protocols on cycling endurance performance. Specifically we explored, for the first time, whether the "dose" of IPC, reflected by either the number of cycles, or the amount of muscle tissue occluded, affects endurance cycling TT performance. We provide evidence that the traditional (4x5-min) occlusion/reperfusion cycles

resulted in the fastest TT times. Our data may support application of a traditional IPC "dose" of cycles, since increasing the "dose" by applying more cycles and reducing the "dose" by applying unilateral IPC, resulted in no further benefit to endurance performance. Furthermore, our study provides evidence that the same magnitude of change in TT time (17 seconds) occurs when exposed to either local or remote application of IPC.

Ischaemic preconditioning, applied using the traditional (4x5-min) inflation/reperfusion cycles [9,20–24], mediated an effect that was an unclear performance improvement in a 375 kJ cycling TT based on a the signal to noise ratio. The improvement of 17 seconds following traditional IPC vs SHAM is marginally below the calculated error and the confidence intervals do not cross zero therefore we are confident that a directional change is present in favor of a worthwhile performance improvement. Furthermore, our observation of a 1.4% performance change is largely in line with previous reports examining the impact of traditional IPC on endurance-type exercise tasks [12], but it is important to emphasise that we included a trained population (natural coefficient of variation of 1.1%); something not commonly observed to date in time-trial based performance tasks, with the exception of competitive swimmers [20,25,26]. The research evidence suggests IPC can improve exercise capacity in recreationally trained participants

[4], but one recent study demonstrated that in highly trained athletes, IPC provided little benefit in improving exercise capacity [27]. Whether a higher aerobic capacity blunts the ergogenic effect of IPC on exercise performance using sports specific tasks remains to be determined.

Importantly, the difference in TT time following a larger "dose", through applying more (8x5-min) cycles in one session, was not deemed substantial enough, when compared to SHAM, to be of benefit. In addition, a smaller "dose" by applying unilateral IPC had little beneficial impact on performance. These results suggest for the first time, that IPC-mediated performance improvements are unlikely amplified by doubling the "traditional" number of IPC cycles. Nevertheless, it is unclear whether an area threshold is present for the "dose-tissue". Whilst no negative impact on TT time was suggested from the magnitude based inference, the lack of additional benefit on exercise performance after the 8x5-min protocol provides support for the 'hyperconditioning' hypothesis, in that too many cycles may negate the beneficial effects of IPC [7].

A recent animal model corroborated these findings and demonstrated four to six cycles yielded cardioprotection, with no further benefit after using eight cycles [28]. Additionally, it was found that when using four cycles, both unilateral and bilateral hind-limb occlusion offered similar cardioprotection [28]. The current study

findings suggest a bilateral "dose", but not unilateral "dose", may result in greater endurance performance; an outcome in line with one previous human study showing bilateral, but not unilateral IPC improved anaerobic sprint cycling performance [8]. Whilst our data is specific to aerobic exercise performance, it may be possible that an "area threshold" i.e. a required amount tissue occlusion, is required to stimulate IPC-induced performance improvements, regardless of intensity [8,29].

Remote IPC can elicit cardio protective effects, comparable to local IPC, possibly as a result of a humoral trigger signal or circulating factor [20]. To date, the comparison between remote and local IPC has not been directly examined in an human performance setting, although both protocols have been previously reported to enhance performance when compared to SHAM [8,9]. In our study, we provide the first direct evidence that local and remote application of IPC resulted in the same TT performance (288 watts, respectively). Whether a systemic pathway contribution towards improved exercise performance occurs, such as a humoral trigger signal or circulating factor similar to that shown with cardioprotection [20] remains to be seen. Interestingly, clinical application of IPC locally or remotely is associated with a comparable protective effect against ischaemia-reperfusion injury in animals and humans [11].

TT performance after the traditional IPC "dose" was accompanied by a lower $\dot{V}O_2$ when compared to SHAM. Our data also reveal a lower TT $\dot{V}O_2$ for the same given workload (288w average) following local, compared to remote IPC. Whilst local IPC application can increase pig skeletal muscle metabolic efficiency under ischaemic conditions [2], it remains unknown whether previously observed local IPC-induced metabolic adaptations [9,30] may have contributed to these findings. Nevertheless, the current data are suggestive that traditional IPC, applied locally, enhances the ability to sustain the same workload for a relatively lower oxygen cost compared to both SHAM and remote IPC, but this does not necessarily relate to clear improvements in power output.

We recorded lactate measurements at each 25% stage of TT performance and found the traditional "dose" of IPC increased blood lactate during exercise when compared to both SHAM and the 8x5-min condition. This finding is somewhat intriguing given that we have previously reported a lower onset of blood lactate accumulation (OBLA) during submaximal exercise following 4x5-min (traditional) bilateral IPC compared to SHAM, hypothesizing greater lactate removal and transportation for uptake [9]. A logical explanation for this apparent contrasting result is that workload in the current cycling TT task markedly exceeds that at OBLA. The increased blood lactate response in the current study following 4x5-

417 min local bilateral IPC, combined with lower $\dot{V}O_2$, could be
418 suggestive of alterations in substrate utilisation, with a proposed
419 heightened anaerobic energy contribution. This was recently
420 inferred by Cruz et al. [31], who demonstrated 4x5-min cycles of IPC
421 improved 60-second sprint cycling performance and lead to an
422 increased skeletal muscle activation during exercise, whilst during
423 recovery produced higher amplitude of blood lactate kinetics and
424 increased excess post-exercise oxygen consumption (EPOC), when
425 compared to SHAM exercise. This, in combination with our data,
426 suggests the potential ergogenic mechanisms relating to IPC-
427 induced metabolic alteration, is likely task and/or intensity specific.
428 The capability of IPC to enhance aerobic exercise capacity [4,29,30], yet
429 have smaller ergogenic effects on fixed-end-point performance [12] is
430 a relationship also observed following the use of nitrate based
431 dietary interventions [32] and might provide some insight into
432 potential mechanisms.

433 A systematic review and meta-analysis [12] recently reported IPC can
434 enhance incremental exercise performance, time to exhaustion task
435 performance, and fixed-end-point task performance by 2.4%, 5.8%
436 and 0.5%, respectively. Additionally, Ferreira et al. [25] stated the
437 estimated performance improvement of IPC was 1.5% based on
438 some previous study findings [9,20,29]. The current observed
439 performance changes (1.4%) are broadly in line with the above

studies, yet the cycling mode we employed was a fixed-end-point task. We further delimited the impact of pacing strategy with rigorous familiarization trials (mean co-efficient of variation in TT time between trials was 1.1% ± 0.8%), and selecting only trained cyclists as participants.

Practical Applications:

IPC is a well-tolerated intervention for the competing individual (table 4). The magnitude of improvement after a bilateral 4x5-min protocol, independent of whether cuffs are placed locally (upper thighs) or remotely (upper arms), lead to improvements in finish time. This conclusion is based on the calculated typical error of our laboratory based test. Given the performance changes in laboratory based tests are different to the field and in competition (e.g. power-velocity relationship on the road is cubic and not linear) this needs to be taken into account when applying these findings to road competition.

Conclusion

Our results suggest the "traditional" protocol of IPC involving 4x5-min occlusion is associated with the fastest TT time compared to SHAM, in a laboratory 375 kJ TT task, aimed to simulate demands of a 16.1 km road TT race. Moreover, by applying different IPC protocols in a within-subject cross-over design, our data suggests no

benefit when increasing the "dose" by doubling the number of cycles or reducing the "dose" via implementing unilateral IPC. Finally, TT performance after IPC appears to be independent of the localization of the cuffs, as IPC applied to the upper limbs resulted in the same TT time.

Acknowledgements: The authors would like to thank the committed participants for giving up their time to undertake physically demanding protocols, in order to obtain the data for this study.

Conflict of Interest: None to declare. Results of the present study do not constitute endorsement by any party and all results are presented clearly, honestly, and without fabrication or falsification.

References:

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74(5):1124-1136.

2. Pang CY, Yang RZ, Zhong A, Xu N, Boyd B, Forrest CR. Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. *Cardiovasc Res*. 1995;29(6):782-788.

3. Kraemer R, Lorenzen J, Kabbani M, et al. Acute effects of remote ischemic preconditioning on cutaneous microcirculation-a controlled prospective cohort study. *BMC Surg*. 2011;11(1):32.

4. de Groot PCE, Thijssen DHJ, Sanchez M, Ellenkamp R, Hopman MTE. Ischemic preconditioning improves maximal performance in humans. *Eur J Appl Physiol*. 2010;108(1):141-146. doi:10.1007/s00421-009-1195-2.

5. Meng R, Asmaro K, Meng L, et al. Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology*. 2012;79(18):1853-1861. doi:10.1212/WNL.0b013e318271f76a.

6. Jones H, Hopkins N, Bailey TG, Green DJ, Cable NT, Thijssen DHJ. Seven-Day Remote Ischemic Preconditioning Improves Local and Systemic Endothelial Function and Microcirculation in Healthy Humans. *Am J Hypertens*. 2014;27(7):918-925. doi:10.1093/ajh/hpu004.

7. Whittaker P, Przyklenk K. From Ischemic Conditioning to "Hyperconditioning": Clinical Phenomenon and Basic Science Opportunity. *Dose-Response*. 2014;12(4):650-663. doi:10.2203/dose-response.14-035.Whittaker.

8. Kraus AS, Pasha EP, Machin DR, Alkatan M, Kloner RA, Tanaka H. Bilateral Upper Limb Remote Ischemic Preconditioning Improves Anaerobic Power. *Open Sports Med J*. 2015;9(1). http://benthamopen.com/ABSTRACT/TOSMJ-9-1. Accessed September 3, 2015.

9. Bailey TG, Jones H, Gregson W, Atkinson G, Cable NT, Thijssen DHJ. Effect of Ischemic Preconditioning on Lactate Accumulation and Running Performance: *Med Sci Sports Exerc*. 2012;44(11):2084-2089. doi:10.1249/MSS.0b013e318262cb17.

10. Barbosa TC, Machado AC, Braz ID, et al. Remote ischemic preconditioning delays fatigue development during handgrip exercise: RIPC improves handgrip performance. *Scand J Med Sci Sports*. 2015;25(3):356-364. doi:10.1111/sms.12229.

11. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic'preconditioning'protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation*. 1993;87(3):893-899.

12. Salvador AF, De Aguiar RA, Lisbôa FD, Pereira KL, Cruz RS de O, Caputo F. Ischemic Preconditioning and Exercise Performance: A Systematic Review and Meta-Analysis. *Int J Sports Physiol Perform*. 2016;11(1):4-14. doi:10.1123/ijspp.2015-0204.

13. Jeukendrup A, Saris WH, Brouns F, Kester AD. A new validated endurance performance test. *Med Sci Sports Exerc*. 1996;28(2):266-270.

14. Sharma V, Cunniffe B, Verma AP, Cardinale M, Yellon D. Characterization of acute ischemia-related physiological responses associated with remote ischemic preconditioning: a randomized controlled, crossover human study. *Physiol Rep*. 2014;2(11):e12200-e12200. doi:10.14814/phy2.12200.

15. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales: *Pain*. 2011;152(10):2399-2404. doi:10.1016/j.pain.2011.07.005.

16. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ*. 1998;316(7139):1236-1238.

17. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. *Int J Sports Physiol Perform*. 2006;1(1):50-57.

18. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2. ed., reprint. New York, NY: Psychology Press; 2009.

19. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3-13. doi:10.1249/MSS.0b013e31818cb278.

20. Jean-St-Michel E, Manlhiot C, Li J, et al. Remote Preconditioning Improves Maximal Performance in Highly Trained Athletes: *Med Sci Sports Exerc*. 2011;43(7):1280-1286. doi:10.1249/MSS.0b013e318206845d.

21. Cruz RS de O, de Aguiar RA, Turnes T, Pereira KL, Caputo F. Effects of ischemic preconditioning on maximal constant-load cycling performance. *J Appl Physiol*. 2015;119(9):961-967. doi:10.1152/japplphysiol.00498.2015.

22. Patterson SD, Bezodis NE, Glaister M, Pattison JR. The Effect of Ischemic Preconditioning on Repeated Sprint Cycling Performance: *Med Sci Sports Exerc*. 2015;47(8):1652-1658. doi:10.1249/MSS.0000000000000576.

23. Kjeld T, Rasmussen MR, Jattu T, Nielsen HB, Secher NH. Ischemic Preconditioning of One Forearm Enhances Static and Dynamic Apnea: *Med Sci Sports Exerc*. 2014;46(1):151-155. doi:10.1249/MSS.0b013e3182a4090a.

24. Paixão R, da Mota G, Marocolo M. Acute Effect of Ischemic Preconditioning is Detrimental to Anaerobic Performance in Cyclists. *Int J Sports Med*. 2014;35(11):912-915. doi:10.1055/s-0034-1372628.

25. Ferreira TN, Sabino-Carvalho JL, Lopes TR, et al. Ischemic Preconditioning and Repeated Sprint Swimming: A Placebo and Nocebo Study. *Med Sci Sports Exerc*. May 2016:1. doi:10.1249/MSS.0000000000000977.

26. Lisbôa FD, Turnes T, Cruz RSO, Raimundo JAG, Pereira GS, Caputo F. The time dependence of the effect of ischemic preconditioning on successive sprint swimming performance. *J Sci Med Sport*. September 2016. doi:10.1016/j.jsams.2016.09.008.

27. Hittinger EA, Maher JL, Nash MS, et al. Ischemic preconditioning does not improve peak exercise capacity at sea level or simulated high altitude in trained male cyclists. *Appl Physiol Nutr Metab*. 2015;40(1):65-71. doi:10.1139/apnm-2014-0080.

28. Johnsen J, Pryds K, Salman R, Løfgren B, Kristiansen SB, Bøtker HE. The remote ischemic preconditioning algorithm: effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. *Basic Res Cardiol*. 2016;111(2). doi:10.1007/s00395-016-0529-6.

29. Crisafulli A, Tangianu F, Tocco F, et al. Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. *J Appl Physiol*. 2011;111(2):530-536. doi:10.1152/japplphysiol.00266.2011.

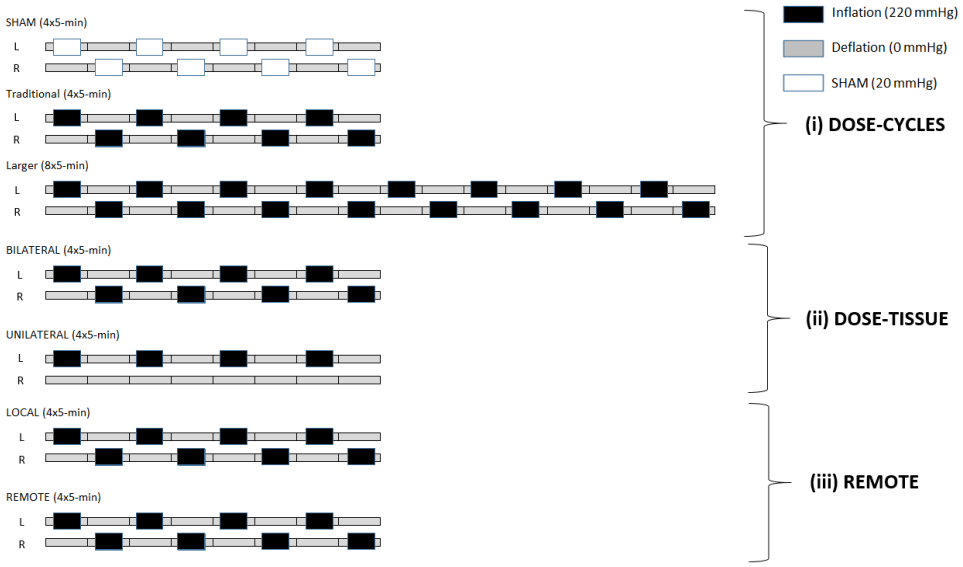
30. Kido K, Suga T, Tanaka D, et al. Ischemic preconditioning accelerates muscle deoxygenation dynamics and enhances exercise endurance during the work-to-work test. *Physiol Rep*. 2015;3(5):e12395-e12395. doi:10.14814/phy2.12395.

31. Cruz RS de O, de Aguiar RA, Turnes T, Salvador AF, Caputo F. Effects of ischemic preconditioning on short-duration cycling performance. *Appl Physiol Nutr Metab Physiol Appl Nutr Metab*. 2016;41(8):825-831. doi:10.1139/apnm-2015-0646.

32. McMahon NF, Leveritt MD, Pavey TG. The Effect of Dietary Nitrate Supplementation on Endurance Exercise Performance in Healthy Adults: A Systematic Review and Meta-Analysis.

Sports Med. September 2016. doi:10.1007/s40279-016-0617-7.

Figures:



627

628

629 Figure 1 – Schematic of different of IPC protocols (i) comparison of
630 dose-cycles (ii) comparison of dose-tissue and (ii) comparison
631 remote. (N.B. traditional dose of IPC was performed once in the
632 experimental design but is shown 3 times on schematic to highlight
633 the comparisons).

634

635

636

637

638

639

640

641

Figure 2a

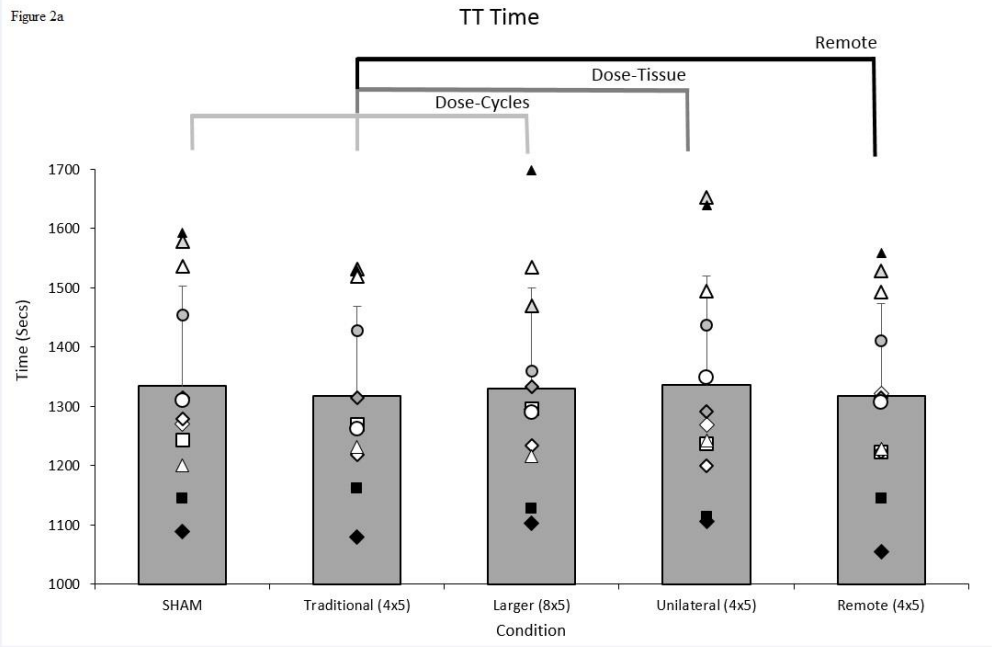
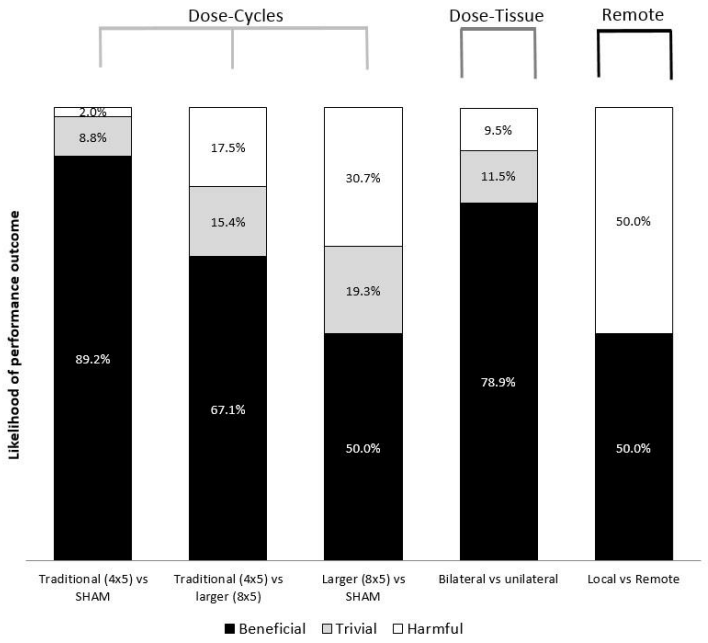


Figure 2b



642

643

644 Figure 2a – Overall TT times (with individual times plotted) for IPC
645 (i) comparison of dose-cycles (ii) comparison of dose-tissue (iii)
646 comparison of remote.

647 Figure 2b – A between-condition representation of the likelihood of
648 "beneficial", "trivial", or "harmful" performance outcome to
649 endurance cycling TT performance.

Tables:

Table 1: The effect of "dose-cycles" on power, heart rate, rate of perceived exertion and $\dot{V}O_2$ following 25%, 50%, 75% and 100% time points during time trial performance.

	Intervention					*P* values	
	Average	0-25%	25-50%	50-75%	75-100%		
Power (watts)							
4x5	288 ± 33	310 ± 38	284 ± 34	275 ± 34	285 ± 32	Condition	0.57
8x5	286 ± 35	307 ± 37	284 ± 35	273 ± 37	281 ± 36	Time	< 0.005
SHAM	285 ± 35	305 ± 38	282 ± 39	273 ± 35	284 ± 35	Condition x time	0.99
Lactate ($mmol.L^{-1}$)							
4x5	11.8 ± 2.8	10.8 ± 3.4	11.8 ± 3.2	12.4 ± 2.9	13.4 ± 2.8*	Condition	0.02*
8x5	11.2 ± 3.1	10.6 ± 3.7	11.2 ± 3.2	11.3 ± 3.3	11.6 ± 3.2*	Time	< 0.005
SHAM	11.4 ± 4.3	10.1 ± 4	10.7 ± 5.1	11.5 ± 4.5	13.2 ± 4.4	Condition x time	0.69
HR (BPM)							
4x5	168 ± 11	158 ± 12	168 ± 11	170 ± 10	173 ± 10	Condition	0.45
8x5	167 ± 13	158 ± 15	166 ± 13	170 ± 13	173 ± 13	Time	< 0.005
SHAM	166 ± 14	154 ± 15	165 ± 14	168 ± 14	171 ± 14	Condition x time	0.96
RPE (Borg scale 6-21)							
4x5	17.7 ± 1.1	16.1 ± 1.6	17.2 ± 1.5	17.7 ± 1.3	19.3 ± 0.9	Condition	0.83
8x5	17.7 ± 1.1	16.6 ± 1.1	17.3 ± 1.3	17.9 ± 1.2	18.8 ± 1.1	Time	< 0.005
SHAM	17.6 ± 1	16.2 ± 1.3	17.1 ± 1.2	17.8 ± 1.4	19 ± 0.9	Condition x time	0.64
V ̇O2 ($ml.kg.min^{-1}$)							
4x5	52.6 ± 4.4	49.8 ± 3.3	54.6 ± 4.8	53.2 ± 5.3	52.8 ± 4.7	Condition	0.08
8x5	52.8 ± 4.3	50.3 ± 3.6	54.8 ± 4.7	53.6 ± 5.3	52.8 ± 4.7	Time	< 0.005
SHAM	53.3 ± 4.4	50.4 ± 3.7	55.6 ± 4.7	54.1 ± 4.8	53.3 ± 4.9	Condition x time	0.1

Table 2: The effect of "dose-tissue" on power, heart rate, rate of perceived exertion and $\dot{V}O_2$ following 25%, 50%, 75% and 100% time points during time trial performance.

	Intervention					*P* values	
	Average	0-25%	25-50%	50-75%	75-100%		
Power (Watts)							
BILATERAL	288 ± 33	310 ± 38	284 ± 34	275 ± 34	285 ± 32	Condition	0.43
UNI	285 ± 38	305 ± 45	282 ± 42	275 ± 36	282 ± 36	Time	< 0.005
						Condition x time	0.75
Lactate ($mmol.L^{-1}$)							
BILATERAL	11.8 ± 2.8	10.7 ± 3.4	11.5 ± 3.2	12 ± 2.7	13.1 ± 2.9	Condition	0.83
UNI	11.7 ± 3.5	10.9 ± 3.9	11.6 ± 4.1	11.8 ± 3.6	12.9 ± 3.7	Time	0.001
						Condition x time	0.1
HR (BPM)							
BILATERAL	168 ± 11	158 ± 12	168 ± 11	170 ± 10	173 ± 10	Condition	0.21
UNI	168 ± 13	158 ± 15	169 ± 13	171 ± 14	173 ± 13	Time	< 0.005
						Condition x time	0.38
RPE (Borg scale 6-21)							
BILATERAL	17.7 ± 1.1	16.1 ± 1.6	17.2 ± 1.5	17.7 ± 1.3	19.3 ± 0.9	Condition	0.44
UNI	17.5 ± 1	16.3 ± 1.2	17.3 ± 1	17.7 ± 1.2	18.9 ± 1	Time	< 0.005
						Condition x time	0.77
V ̇O2 ($ml.kg.min^{-1}$)							
BILATERAL	52.6 ± 4.2	49.8 ± 3.4	54.6 ± 4.5	53.2 ± 5	52.8 ± 4.6	Condition	0.26
UNI	52.5 ± 5.6	49 ± 4.5	54 ± 6.2	53.8 ± 6.1	53.3 ± 6	Time	< 0.005
						Condition x time	0.06

Table 3: The effect of "remote" IPC on power, heart rate, rate of perceived exertion and $\dot{V}O_2$ at 25%, 50%, 75% and 100% time points during time trial performance.

	Intervention					*P* values	
	Average	0-25%	25-50%	50-75%	75-100%		
Power (Watts)							
LOCAL	288 ± 33	310 ± 38	284 ± 34	275 ± 34	285 ± 32	Condition	0.8
REMOTE	288 ± 35	308 ± 39	286 ± 33	277 ± 35	286 ± 40	Time	< 0.005
						Condition x time	0.94
Lactate ($mmol.L^{-1}$)							
LOCAL	11.8 ± 3	10.7 ± 3.4	11.5 ± 3.2	12 ± 2.7	13.1 ± 3	Condition	0.24
REMOTE	11.4 ± 5	9.8 ± 3.9	11.2 ± 4	11.4 ± 4	13.4 ± 6.1	Time	< 0.005
						Condition x time	0.93
HR (BPM)							
LOCAL	168 ± 11	158 ± 12	168 ± 11	170 ± 10	173 ± 10	Condition	0.56
REMOTE	167 ± 14	158 ± 15	168 ± 14	171 ± 13	173 ± 13	Time	< 0.005
						Condition x time	0.41
RPE (Borg scale 6-21)							
LOCAL	17.7 ± 1.1	16.1 ± 1.6	17.2 ± 1.5	17.7 ± 1.3	19.3 ± 0.9	Condition	0.72
REMOTE	17.6 ± 1.1	16.5 ± 1.2	17.3 ± 1.4	17.6 ± 1.2	19 ± 1	Time	< 0.005
						Condition x time	0.57
$V\dot{O}2$ ($ml.kg.min^{-1}$)							
LOCAL	52.6 ± 3.8	49.8 ± 3.1	54.6 ± 4.1	53.2 ± 4.6*****	52.8 ± 4.4	Condition	0.04*
REMOTE	53.4 ± 4.3	50.4 ± 3.3	55.1 ± 4.6	54.5 ± 5*	53.7 ± 5	Time	< 0.005
						Condition x time	0.36

682

683

684 Table 4: Perceived discomfort of IPC and SHAM interventions.

	Perceived discomfort of condition (ratings 0-10)					*Mean discomfort rating*
	Average	0-10 min	10-20 min	20-30 min	30-40 min	
Traditional 4x5 IPC (legs)	3.7 ± 1.2	4.5 ± 1.5	3.5 ± 1.1	3.5 ± 1.1	3.4 ± 1.1	Light to moderate
Larger 8x5 cycles	3.6 ± 1.7	3.9 ± 1.8	3.5 ± 1.6	3.3 ± 1.8	3.5 ± 1.8	Light to moderate
Unilateral 4x5 IPC	3.1 ± 1.5	3.5 ± 1.9	3.1 ± 1.5	2.8 ± 1.3	2.8 ± 1.5	Light to moderate
Remote 4x5 IPC (arms)	3.7 ± 2.1	4.1 ± 2	3.6 ± 2	3.7 ± 2	3.4 ± 2.3	Light to moderate
SHAM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	No discomfort

685

686

687

688