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

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1 2	Is There an Optimal Ischaemic Preconditioning Dose to Improve Cycling Performance?
3	Scott Cocking ^{1, 2} ,
4	Mathew G. Wilson ^{1,2}
5	David Nichols ^{1, 2}
6	N. Timothy Cable ^{2, 5}
7	Daniel J. Green ² , ³
8	Dick H. J. Thijssen ^{2,4}
9 10	Helen Jones ²
11 12	¹ Athlete Health and Performance Research Centre, <i>Aspetar</i> <i>Orthopaedic and Sports Medicine Hospital</i> , Doha, Qatar.
13 14	² Research Institute for Sport and Exercise Science, <i>Liverpool John</i> <i>Moores University</i> , UK
15 16	³ School of Sports Science, Exercise and Health, The University of Western Australia, Crawley, Perth, Western Australia, Australia
17 18	⁴ Radboud Institute of Health Sciences, Department of Physiology, <i>Radboud University Medical Center</i> , the Netherlands
19	⁵ Department of Sport Science, Aspire Academy, Doha, Qatar
20	
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26	Author for correspondence:
27 28 29 30 31 32 33	Prof Helen Jones, Research Institute of Sports and Exercise Science, Liverpool John Moores University, Tom Reilly Building, Byrom Street, Liverpool, L3 3AF <u>h.jones1@ljmu.ac.uk</u> <i>TEL:</i> +441519046270 DISCLOSURE: No conflicts of interest
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34 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.

41 *METHODS*: Twelve cyclists performed five different IPC protocols 42 30-min prior to a blinded 375 kJ cycling time trial (TT) in a 43 laboratory. Responses to traditional IPC (4x5-min legs) were 44 compared to: i. 8x5-min legs and SHAM ("dose-cycles"), ii. 4x5-45 min unilateral legs ("dose-tissue"), and *iii*. 4x5-min arms 46 ("remote"). RPE and blood lactate were recorded at each 25% TT 47 completion. Power (watts), heart rate (bpm), and $\dot{V}O_2$ (ml.kg.min⁻¹) 48 were measured continuously throughout TT's. Magnitude based 49 inference statistics were employed to compare variable differences 50 to the minimal practically important difference.

51 *RESULTS:* Traditional IPC was associated with a 17 (0, 34) secs 52 faster TT time compared to SHAM. Applying more "dose-cycles" 53 (8x5-min) had no impact on performance. Traditional IPC was 54 associated with "likely trivial" higher blood lactate and "possibly 55 beneficial" lower $\dot{V}O_2$ responses vs. SHAM. Unilateral IPC was 56 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].

59 *CONCLUSION:* The traditional 4x5-min (local or remote) IPC 60 stimulus resulted in the fastest TT time compared to SHAM, there 61 was no benefit of applying a greater number of cycles or employing 62 unilateral IPC.

63 Key words: Exercise, Occlusion, Ischaemia, Time Trial, Endurance64

65 Introduction

66 Ischaemic preconditioning (IPC) refers to the phenomenon whereby 67 3-4 brief periods of ischaemia, followed by tissue reperfusion, confers subsequent tissue protection against ischaemic insult¹. IPC 68 69 can be applied remotely by placing a blood pressure cuff around a 70 limb and inflating to supra-systolic pressure. Studies have generally 71 employed remote IPC in clinical populations relating to cardio-72 protection, but there is accumulating evidence that remote IPC can 73 impact on other organs (e.g. skeletal muscle), and vascular beds to facilitate increased blood flow ^{2,3}. These finding have resulted in the 74 75 application of IPC to determine its efficacy as a potential pre-76 exercise priming strategy.

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

79 following a 3x5-min bilateral leg cuff inflation (220 mmHg) protocol⁴. A "traditional" IPC protocol consists of 3x5- or 4x5-min 80 81 bouts of occlusion. More recently, studies have separately employed 82 alternative IPC protocols (altering the number of IPC cycles, tissue 83 occlusion area, and cuff location), with the aim of observing greater 84 performance and clinical outcomes. There are now (pre)clinical studies providing evidence for a "dose"-dependency, where 85 86 repeated daily IPC improves (cerebro)vascular function and clinical outcomes ^{5,6}. Nonetheless, a potential 'hyper-conditioning' effect 87 from excessive cycles of IPC cannot be excluded ⁷. Corroborating 88 the "dose"-hypothesis, recent work suggests that bilateral, but not 89 90 unilateral cuff inflation leads to improved exercise performance⁸. 91 Finally, most studies to date have opted for cuff positioning directly on the exercising limb ⁹, but cuff placement on remote, non-92 exercising limbs has also been performed ¹⁰ to examine a systemic 93 94 effect. In line with clinical observations in the protection of organs 95 against ischaemic injury, local or remote application of IPC may 96 induce comparable benefits ^{2,11}.

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

102 Interestingly, no study has directly compared the capacity of distinct 103 IPC protocols with the aim of electing greater performance 104 improvement. Therefore, the aim of this study was to examine 105 whether the (i) number IPC cycles (i.e. "dose-cycles"), (ii) the 106 amount of muscle mass occluded ("dose-tissue"), and (iii) the 107 application of IPC to either local or remote limbs ("remote") offers 108 greater improvements to endurance cycling performance.

109 *Methods*

110 **Participants**

111 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 112 113 recruited. Participants were undertaking regular weekly training 114 sessions (5 \pm 3 sessions) and mean weekly training volume was 8 \pm 4 115 hours. The mean training experience was 9±8 years. Following 116 verbal and written explanation of procedures, all participants 117 provided written informed consent. Physical Activity Readiness 118 Questionnaires were administered to ensure no participant had any 119 health implications that would prevent participation. All individuals 120 refrained from exercise and alcohol consumption 24 hours, and 121 consumption of caffeine at least 6 hours, respectively prior to all 122 laboratory visits. The study was approved by the local Ethics Committee. 123

124 Research Design

125 The study was divided into three comparisons as illustrated in figure 126 1. All participants completed a maximal graded cycling test and at 127 least two familiarization TT. Prior to commencement of the five 128 experimental cycling TT's, an IPC protocol was administered. A 129 traditional (4x5-min) IPC protocol was compared firstly to SHAM, 130 and a larger (8x5-min) IPC protocol for the "dose-cycles" 131 comparison. Whilst it was compared to a unilateral (4x5-min) IPC 132 protocol for the "dose-tissue" comparison. Finally, to assess the 133 importance of cuff placement, a 4x5-min bilateral IPC protocol was 134 applied to the non-exercising upper limb for the "remote" 135 comparison.

136 Experimental Protocol

137 In a randomized, counterbalanced, crossover study, participants 138 reported to the laboratory at the same time of day on five separate 139 occasions, at least 4 days apart, receiving a different pre-exercise 140 IPC protocol during each visit. Following each IPC protocol, a 20-141 minute rest period, and a standardized warm up was performed 142 before the completion of a 375 kilojoule (kJ) cycling time trial (TT). 143 The TT was intended to simulate the demands of a 16.1 km TT. 144 During each TT, heart rate and oxygen uptake ($\dot{V}O_2$) was measured 145 continuously, whilst blood lactate and rate of perceived of exertion

146 (RPE) was recorded at every 25% completed of the TT kilojoule147 target.

148 Measurements

Assessment of maximal oxygen uptake ($\dot{V}O_{2max}$). At least 7 days prior 149 150 to the first familiarisation trial, participants performed a continuous 151 incremental step test on an electromagnetically braked cycle 152 ergometer (SRM, Julich, Germany) to determine lactate threshold 153 and $\dot{V}O_{2max}$. The incremental protocol consisted of 3-minute cycling 154 stages, commencing at 95 watts (W) and increasing 35W until 155 volitional exhaustion occurred. Blood lactate concentration was 156 obtained via finger prick capillary sampling using a safety lancet 157 (BD Microtainer® Contact-Activated Lancet) after administration 158 of a disposable sterile isoprophyl alcohol swab (China MEHECO 159 Co., Ltd.). Blood was collected into a sodium-heparinized blood gas 160 capillary tube (Marienfeld Superior, Germany) and immediately 161 analysed in duplicate (ABL90 FLEX, Radiometer Medical ApS, 162 Denmark) during the last 30 seconds of each incremental stage. 163 Throughout the incremental cycling test, breath-by-breath expired 164 gases were monitored for oxygen consumption, ventilation and 165 respiratory exchange ratio (RER) (MasterScreenTM CPX, 166 Carefusion, Germany) and the highest 30-second average was taken 167 3 consecutive 10-second bins from subsequently to determine $\dot{V}O_{2max}$. Heart rate (HR) was also monitored continuously 168

(Polar H1, Kempele, Finland). W_{max} was calculated from the last
completed workload, plus the fraction of time spent in the final noncompleted 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.

177 IPC protocols. For the IPC and SHAM trials, 13.5 cm wide cuffs 178 were used. Participants lay in the supine position and cuff inflation 179 pressure was set at a standardized pressure (220mmHg) in all IPC conditions with the aim of preventing arterial inflow ¹⁴ and 20mmHg 180 181 in SHAM (i.e. cuffs were placed but only inflated to 20mmHg) with 182 the use of an automatic rapid cuff inflator (Hokanson, Washington, 183 USA). Subsequently, cuffs were deflated for 5 minutes, allowing 184 reperfusion. This process was repeated four times in all protocols 185 except for the "dose-cycles" protocol where 8 cycles were used 186 (Figure 1). For IPC on the leg, the cuff was placed (unilaterally or 187 bilaterally) on the most proximal portions of the upper thigh (distal 188 to the inguinal fold). For remote IPC, cuffs were placed on the most 189 proximal portions of the upper arms. Each participant gave a 190 "perceived discomfort" rating at four time points (every 25%) throughout the IPC or SHAM protocols. The discomfort rating was 191

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.

195 375 kJ TT. After 20 minutes of rest following cessation of 196 IPC/SHAM, a capillary blood lactate sample was obtained from the 197 finger and analysed for resting lactate levels (ABL90 FLEX, 198 Radiometer Medical ApS, Denmark). Participants then completed a 199 standardized warm up on an electromagnetically braked cycle 200 ergometer (SRM, Julich, Germany). The warm up lasted 201 approximately 10 minutes (5-min at 100W, 2-min at 150W, [15-secs 202 at W_{max} , 30-secs at 150W, repeat x3], 45-secs at 150W). Once the 203 flywheel had completely stopped turning, the SRM clock was reset 204 to zero and a 375 kJ TT was performed (exactly 35 minutes after 205 completion of IPC in all trials). Participants were instructed to 206 produce a maximum effort throughout TT's, but were blinded to 207 power output, elapsed time and HR. Breath-by-breath expired gases 208 and HR were measured continuously, while RPE and blood lactate 209 measurements were acquired at 25%, 50%, 75% and 100% time 210 points (all described previously). Participants were notified once 211 they had completed each quarter of the TT and when they had 30 kJ 212 of work remaining. No encouragement or feedback was given 213 throughout any trial.

214 Statistical Analysis

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

238 between each comparison, together with its uncertainty, the 239 probability (percent chances) that the true population effect was 240 beneficial (>SWC), harmful (>SWC with opposite sign), or trivial 241 (within \pm SWC) was calculated ¹⁸. Using mechanistic inferences, 242 qualitative probabilistic terms for benefit were assigned to each 243 effect using the following scale; <0.5%, most unlikely or almost 244 certainly not; 0.5 to 5%, very unlikely; 5 to 25%, unlikely or 245 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 246 247 unclear effect is possibly beneficial (>25%) with an unacceptable 248 risk of harm (>0.5%) and an odds ratio for benefit:harm of <66 249 interpreted from current recommendations; all other effects are 250 clear. Data that were lower than the typical error (noise > signal) for 251 TT performance were interpreted as "unclear" and reported with the 252 confidence limits within the text and in figure 2.

253 *Results*

254 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%).

266 $\dot{V}O_2$: $\dot{V}O_2$ was 0.99 ml.kg.min⁻¹ (-1.7, -0.3 ml.kg.min⁻¹) lower 267 following traditional IPC compared to SHAM, interpreted as 268 "possibly beneficial" (beneficial 59%, trivial 41%, harmful 0%; 269 P=0.03). A "likely trivial" difference was evident between 270 traditional IPC and the 8x5-min protocol [0.51 ml.kg.min⁻¹(-1.2, 0.2 271 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 8x5min 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).

283 Dose-tissue

- 284 TT Time: Traditional bilateral IPC resulted in an 18 secs (-11, 48
- secs, P=0.29; Figure 2) faster TT performance than unilateral IPC.
- 286 Nevertheless, this change was interpreted as "unclear" (beneficial
- 287 78%, trivial 12%, harmful 10%).
- 288 $\dot{V}O_2$: The lower resultant $\dot{V}O_2$ following traditional IPC compared 289 to unilateral IPC [0.8 ml.kg.min⁻¹; (-2, 0.4 ml.kg.min⁻¹); (beneficial 290 45%, trivial 54%, harmful 1%) *P*=0.26)] was interpreted as 291 "possibly trivial". The time-dependent effect (Table 2), was not 292 different between the 2 trials.
- 293 *Lactate*: Blood lactate increased throughout TT performance, with 294 highest values during 4th quarter (Table 2). The mean blood lactate 295 difference of 0.05 mmol.L⁻¹ (-1.3, 1.4 mmol.L⁻¹); (beneficial 11%, 296 trivial 81%, harmful 9%; P=0.95) between protocols was 297 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).

301 **Remote**

302 TT time: The comparison of traditional IPC and remote IPC resulted303 in a negligible difference in mean TT time [0 secs (-16, 16 secs;

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

306 $\dot{V}O_2$: $\dot{V}O_2$ was 1.1 ml.kg.min⁻¹ (-1.9, -0.2 ml.kg.min⁻¹; (beneficial 307 71%, trivial 29%, harmful 0%) *P*=0.04) lower following the 308 traditional protocol compared to remote IPC, interpreted as a 309 "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).

319 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.

333 Ischaemic preconditioning, applied using the traditional (4x5-min) 334 inflation/reperfusion cycles ^{9,20–24}, mediated an effect that was an 335 unclear performance improvement in a 375 kJ cycling TT based on 336 a the signal to noise ratio. The improvement of 17 seconds following 337 traditional IPC vs SHAM is marginally below the calculated error 338 and the confidence intervals do not cross zero therefore we are 339 confident that a directional change is present in favor of a 340 worthwhile performance improvement. Furthermore, our 341 observation of a 1.4% performance change is largely in line with 342 previous reports examining the impact of traditional IPC on 343 endurance-type exercise tasks ¹², but it is important to emphasise 344 that we included a trained population (natural coefficient of 345 variation of 1.1%); something not commonly observed to date in 346 time-trial based performance tasks, with the exception of competitive swimmers ^{20,25,26}. The research evidence suggests IPC 347 348 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.

354 Importantly, the difference in TT time following a larger "dose", 355 through applying more (8x5-min) cycles in one session, was not 356 deemed substantial enough, when compared to SHAM, to be of 357 benefit. In addition, a smaller "dose" by applying unilateral IPC had 358 little beneficial impact on performance. These results suggest for the 359 first time, that IPC-mediated performance improvements are 360 unlikely amplified by doubling the "traditional" number of IPC 361 cycles. Nevertheless, it is unclear whether an area threshold is 362 present for the "dose-tissue". Whilst no negative impact on TT time 363 was suggested from the magnitude based inference, the lack of 364 additional benefit on exercise performance after the 8x5-min 365 protocol provides support for the 'hyperconditioning' hypothesis, in 366 that too many cycles may negate the beneficial effects of IPC 7 .

367 A recent animal model corroborated these findings and 368 demonstrated four to six cycles yielded cardioprotection, with no 369 further benefit after using eight cycles ²⁸. Additionally, it was found 370 that when using four cycles, both unilateral and bilateral hind-limb 371 occlusion offered similar cardioprotection ²⁸. The current study 372 findings suggest a bilateral "dose", but not unilateral "dose", may 373 result in greater endurance performance; an outcome in line with one 374 previous human study showing bilateral, but not unilateral IPC improved anaerobic sprint cycling performance⁸. Whilst our data is 375 specific to aerobic exercise performance, it may be possible that an 376 377 "area threshold" i.e. a required amount tissue occlusion, is required 378 to stimulate IPC-induced performance improvements, regardless of intensity^{8,29}. 379

380 Remote IPC can elicit cardio protective effects, comparable to local 381 IPC, possibly as a result of a humoral trigger signal or circulating 382 factor ²⁰. To date, the comparison between remote and local IPC has 383 not been directly examined in an human performance setting, 384 although both protocols have been previously reported to enhance performance when compared to SHAM^{8,9}. In our study, we provide 385 386 the first direct evidence that local and remote application of IPC 387 resulted in the same TT performance (288 watts, respectively). 388 Whether a systemic pathway contribution towards improved 389 exercise performance occurs, such as a humoral trigger signal or circulating factor similar to that shown with cardioprotection²⁰ 390 391 remains to be seen. Interestingly, clinical application of IPC locally 392 or remotely is associated with a comparable protective effect against ischaemia-reperfusion injury in animals and humans¹¹. 393

394 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 395 396 lower TT $\dot{V}O_2$ for the same given workload (288w average) 397 following local, compared to remote IPC. Whilst local IPC 398 application can increase pig skeletal muscle metabolic efficiency 399 under ischaemic conditions², it remains unknown whether 400 previously observed local IPC-induced metabolic adaptations ^{9,30} 401 may have contributed to these findings. Nevertheless, the current 402 data are suggestive that traditional IPC, applied locally, enhances 403 the ability to sustain the same workload for a relatively lower 404 oxygen cost compared to both SHAM and remote IPC, but this does 405 not necessarily relate to clear improvements in power output.

406 We recorded lactate measurements at each 25% stage of TT 407 performance and found the traditional "dose" of IPC increased 408 blood lactate during exercise when compared to both SHAM and the 409 8x5-min condition. This finding is somewhat intriguing given that 410 we have previously reported a lower onset of blood lactate 411 accumulation (OBLA) during submaximal exercise following 4x5-412 min (traditional) bilateral IPC compared to SHAM, hypothesizing 413 greater lactate removal and transportation for uptake ⁹. A logical 414 explanation for this apparent contrasting result is that workload in 415 the current cycling TT task markedly exceeds that at OBLA. The 416 increased blood lactate response in the current study following 4x5-

min local bilateral IPC, combined with lower VO₂, could be 417 418 suggestive of alterations in substrate utilisation, with a proposed heightened anaerobic energy contribution. This was recently 419 inferred by Cruz et al. ³¹, who demonstrated 4x5-min cycles of IPC 420 421 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 compared to SHAM exercise. This, in combination with our data, 425 suggests the potential ergogenic mechanisms relating to IPC-426 427 induced metabolic alteration, is likely task and/or intensity specific. The capability of IPC to enhance aerobic exercise capacity ^{4,29,30}, yet 428 429 have smaller ergogenic effects on fixed-end-point performance ¹² is 430 a relationship also observed following the use of nitrate based dietary interventions ³² and might provide some insight into 431 432 potential mechanisms.

A systematic review and meta-analysis ¹² recently reported IPC can enhance incremental exercise performance, time to exhaustion task performance, and fixed-end-point task performance by 2.4%, 5.8% and 0.5%, respectively. Additionally, Ferreira et al. ²⁵ stated the estimated performance improvement of IPC was 1.5% based on some previous study findings ^{9,20,29}. The current observed 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 $1.1\% \pm 0.8\%$), and selecting only trained cyclists as participants.

445 **Practical Applications:**

446 IPC is a well-tolerated intervention for the competing individual 447 (table 4). The magnitude of improvement after a bilateral 4x5-min 448 protocol, independent of whether cuffs are placed locally (upper 449 thighs) or remotely (upper arms), lead to improvements in finish 450 time. This conclusion is based on the calculated typical error of our 451 laboratory based test. Given the performance changes in laboratory 452 based tests are different to the field and in competition (e.g. power-453 velocity relationship on the road is cubic and not linear) this needs 454 to be taken into account when applying these findings to road 455 competition.

456 Conclusion

Our results suggest the "traditional" protocol of IPC involving 4x5min 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.

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471 **Conflict of Interest:** None to declare. Results of the present study

472 do not constitute endorsement by any party and all results are

473 presented clearly, honestly, and without fabrication or falsification.

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



Figure 2b



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

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

Tables:

651Table 1: The effect of "dose-cycles" on power, heart rate, rate of652perceived exertion and $\dot{V}O_2$ following 25%, 50%, 75% and 100%653time 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

660 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
LINU	52.5 ± 5.6	49 ± 4.5	54 ± 6.2	53.8 ± 6.1	53.3 ± 6	Time	< 0.005
UNI							

671Table 3: The effect of "remote" IPC on power, heart rate, rate of672perceived exertion and $\dot{V}O_2$ at 25%, 50%, 75% and 100% time673points 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 O2 (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	$54.5 \pm 5^{*}$	53.7 ± 5	Time	< 0.005
						Condition x time	0.36
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Table 4: Perceived discomfort of IPC and SHAM interventions.

		Pe	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
685	SHAM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	No discomfort
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