

1 Is There an Optimal Ischaemic Preconditioning Dose to Improve
2 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 Helen Jones ²

10

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

13 ² Research Institute for Sport and Exercise Science, *Liverpool John*
14 *Moores University*, UK

15 ³School of Sports Science, Exercise and Health, The University of
16 Western Australia, Crawley, Perth, Western Australia, Australia

17 ⁴Radboud Institute of Health Sciences, Department of Physiology,
18 *Radboud University Medical Center*, the Netherlands

19 ⁵Department of Sport Science, Aspire Academy, Doha, Qatar

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26 **Author for correspondence:**

27 Prof Helen Jones, Research Institute of Sports and Exercise
28 Science, Liverpool John Moores University, Tom Reilly Building,
29 Byrom Street, Liverpool, L3 3AF

30 h.jones1@ljmu.ac.uk

31 *TEL:* +441519046270

32

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34 Abstract

35 *INTRODUCTION:* Ischaemic preconditioning (IPC) may enhance
36 endurance performance. No previous study has directly compared
37 distinct IPC protocols for optimal benefit. The aim of this study was
38 to determine whether a specific IPC protocol (i.e. number of cycles,
39 amount of muscle tissue, and local vs remote occlusion) elicits
40 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

57 bilateral (“dose-tissue”). TT times following remote and local IPC
58 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, Endurance
64

65 *Introduction*

66 Ischaemic preconditioning (IPC) refers to the phenomenon whereby
67 3-4 brief periods of ischaemia, followed by tissue reperfusion,
68 confers subsequent tissue protection against ischaemic insult ¹. IPC
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
74 facilitate increased blood flow ^{2,3}. These finding have resulted in the
75 application of IPC to determine its efficacy as a potential pre-
76 exercise priming strategy.

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

79 following a 3x5-min bilateral leg cuff inflation (220 mmHg)
80 protocol ⁴. A “traditional” IPC protocol consists of 3x5- or 4x5-min
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
85 studies providing evidence for a “dose”-dependency, where
86 repeated daily IPC improves (cerebro)vascular function and clinical
87 outcomes ^{5,6}. Nonetheless, a potential ‘hyper-conditioning’ effect
88 from excessive cycles of IPC cannot be excluded ⁷. Corroborating
89 the “dose”-hypothesis, recent work suggests that bilateral, but not
90 unilateral cuff inflation leads to improved exercise performance ⁸.
91 Finally, most studies to date have opted for cuff positioning directly
92 on the exercising limb ⁹, but cuff placement on remote, non-
93 exercising limbs has also been performed ¹⁰ to examine a systemic
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,
112 78±4 kg; height, 179±6 cm; $\dot{V}O_{2max}$, 59±4 ml.kg⁻¹.min⁻¹) were
113 recruited. Participants were undertaking regular weekly training
114 sessions (5±3 sessions) and mean weekly training volume was 8±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
123 Committee.

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 kilojoule
147 target.

148 **Measurements**

149 *Assessment of maximal oxygen uptake ($\dot{V}O_{2max}$).* At least 7 days prior
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) (MasterScreen™ CPX,
166 Carefusion, Germany) and the highest 30-second average was taken
167 from 3 consecutive 10-second bins to subsequently
168 determine $\dot{V}O_{2max}$. Heart rate (HR) was also monitored continuously

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

172 *Familiarisation.* At least 2 familiarisation trials were undertaken
173 prior to the first experimental TT to ensure performance was
174 reliable. Data from familiarisation sessions revealed a mean
175 coefficient of variation (CoV) of 1.06% which was deemed to be
176 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
180 conditions with the aim of preventing arterial inflow ¹⁴ and 20mmHg
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%)
191 throughout the IPC or SHAM protocols. The discomfort rating was

192 established using a Numerical Rating Scale (NRS) ranging from 0
193 (no discomfort) to 10 (maximum discomfort) and are included for
194 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 calculated¹⁸. 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
246 to 99.5%, very likely; >99.5%, most likely or almost certainly¹⁸. An
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**

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

261 TT time compared to traditional IPC (4x5-min) [13 secs (-19, 44
262 secs); $P=0.49$, (beneficial 67%, trivial 15%, harmful 18%)] Figure
263 2]. The effect between IPC with 8x5-min cycles and SHAM on
264 exercise performance was interpreted as “unclear” (beneficial 50%,
265 trivial 19%, harmful 31%).

266 $\dot{V}O_2$: $\dot{V}O_2$ was $0.99 \text{ ml.kg.min}^{-1}$ (-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 [$0.51 \text{ ml.kg.min}^{-1}$ (-1.2, 0.2
271 ml.kg.min^{-1}); (beneficial 17%, trivial 83%, harmful 0%) $P=0.25$].

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

279 Power / HR / RPE: HR and RPE increased significantly across time
280 ($P<0.05$), whilst power was highest during the 1st quarter. No
281 further differences were evident for power, HR, or RPE between
282 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
285 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”.

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

301 **Remote**

302 TT time: The comparison of traditional IPC and remote IPC resulted
303 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 $1.1 \text{ ml.kg.min}^{-1}$ ($-1.9, -0.2 \text{ ml.kg.min}^{-1}$; (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.

310 Lactate: Blood lactate increased throughout both TT performances,
311 with highest values observed during 4th quarter (Table 3). A mean
312 blood lactate difference of 0.2 mmol.L^{-1} occurred ($-1.2, 1.6 \text{ mmol.L}^{-1}$
313 1 ; $P=0.8$) between both protocols, interpreted as an “unclear”
314 difference (beneficial 18%, trivial 74%, harmful 8%).

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

319 *Discussion*

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

326 resulted in the fastest TT times. Our data may support application of
327 a traditional IPC “dose” of cycles, since increasing the “dose” by
328 applying more cycles and reducing the “dose” by applying unilateral
329 IPC, resulted in no further benefit to endurance performance.
330 Furthermore, our study provides evidence that the same magnitude
331 of change in TT time (17 seconds) occurs when exposed to either
332 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
347 competitive swimmers ^{20,25,26}. The research evidence suggests IPC
348 can improve exercise capacity in recreationally trained participants

349 ⁴, but one recent study demonstrated that in highly trained athletes,
350 IPC provided little benefit in improving exercise capacity ²⁷.
351 Whether a higher aerobic capacity blunts the ergogenic effect of IPC
352 on exercise performance using sports specific tasks remains to be
353 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
375 improved anaerobic sprint cycling performance ⁸. Whilst our data is
376 specific to aerobic exercise performance, it may be possible that an
377 “area threshold” i.e. a required amount tissue occlusion, is required
378 to stimulate IPC-induced performance improvements, regardless of
379 intensity ^{8,29}.

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
385 performance when compared to SHAM ^{8,9}. In our study, we provide
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
390 circulating factor similar to that shown with cardioprotection ²⁰
391 remains to be seen. Interestingly, clinical application of IPC locally
392 or remotely is associated with a comparable protective effect against
393 ischaemia-reperfusion injury in animals and humans ¹¹.

394 TT performance after the traditional IPC “dose” was accompanied
395 by a lower $\dot{V}O_2$ when compared to SHAM. Our data also reveal a
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 conditions ², 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-

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 performance¹² is
430 a relationship also observed following the use of nitrate based
431 dietary interventions³² and might provide some insight into
432 potential mechanisms.

433 A systematic review and meta-analysis¹² 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

440 studies, yet the cycling mode we employed was a fixed-end-point
441 task. We further delimited the impact of pacing strategy with
442 rigorous familiarization trials (mean co-efficient of variation in TT
443 time between trials was $1.1\% \pm 0.8\%$), and selecting only trained
444 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**

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

462 benefit when increasing the “dose” by doubling the number of
463 cycles or reducing the “dose” via implementing unilateral IPC.
464 Finally, TT performance after IPC appears to be independent of the
465 localization of the cuffs, as IPC applied to the upper limbs resulted
466 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.

474 **References:**

475

- 476 1. Murry CE, Jennings RB, Reimer KA. Preconditioning with
477 ischemia: a delay of lethal cell injury in ischemic
478 myocardium. *Circulation*. 1986;74(5):1124-1136.
- 479 2. Pang CY, Yang RZ, Zhong A, Xu N, Boyd B, Forrest CR.
480 Acute ischaemic preconditioning protects against skeletal
481 muscle infarction in the pig. *Cardiovasc Res*. 1995;29(6):782-
482 788.
- 483 3. Kraemer R, Lorenzen J, Kabbani M, et al. Acute effects of
484 remote ischemic preconditioning on cutaneous
485 microcirculation-a controlled prospective cohort study. *BMC*
486 *Surg*. 2011;11(1):32.
- 487 4. de Groot PCE, Thijssen DHJ, Sanchez M, Ellenkamp R,
488 Hopman MTE. Ischemic preconditioning improves maximal
489 performance in humans. *Eur J Appl Physiol*.
490 2010;108(1):141-146. doi:10.1007/s00421-009-1195-2.

- 491 5. Meng R, Asmaro K, Meng L, et al. Upper limb ischemic
492 preconditioning prevents recurrent stroke in intracranial
493 arterial stenosis. *Neurology*. 2012;79(18):1853-1861.
494 doi:10.1212/WNL.0b013e318271f76a.
- 495 6. Jones H, Hopkins N, Bailey TG, Green DJ, Cable NT,
496 Thijssen DHJ. Seven-Day Remote Ischemic Preconditioning
497 Improves Local and Systemic Endothelial Function and
498 Microcirculation in Healthy Humans. *Am J Hypertens*.
499 2014;27(7):918-925. doi:10.1093/ajh/hpu004.
- 500 7. Whittaker P, Przyklenk K. From Ischemic Conditioning to
501 “Hyperconditioning”: Clinical Phenomenon and Basic
502 Science Opportunity. *Dose-Response*. 2014;12(4):650-663.
503 doi:10.2203/dose-response.14-035.Whittaker.
- 504 8. Kraus AS, Pasha EP, Machin DR, Alkatan M, Kloner RA,
505 Tanaka H. Bilateral Upper Limb Remote Ischemic
506 Preconditioning Improves Anaerobic Power. *Open Sports
507 Med J*. 2015;9(1).
508 <http://benthamopen.com/ABSTRACT/TOSMJ-9-1>. Accessed
509 September 3, 2015.
- 510 9. Bailey TG, Jones H, Gregson W, Atkinson G, Cable NT,
511 Thijssen DHJ. Effect of Ischemic Preconditioning on Lactate
512 Accumulation and Running Performance: *Med Sci Sports
513 Exerc*. 2012;44(11):2084-2089.
514 doi:10.1249/MSS.0b013e318262cb17.
- 515 10. Barbosa TC, Machado AC, Braz ID, et al. Remote ischemic
516 preconditioning delays fatigue development during handgrip
517 exercise: RIPC improves handgrip performance. *Scand J Med
518 Sci Sports*. 2015;25(3):356-364. doi:10.1111/sms.12229.
- 519 11. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P.
520 Regional ischemic preconditioning protects remote virgin
521 myocardium from subsequent sustained coronary occlusion.
522 *Circulation*. 1993;87(3):893-899.
- 523 12. Salvador AF, De Aguiar RA, Lisbôa FD, Pereira KL, Cruz
524 RS de O, Caputo F. Ischemic Preconditioning and Exercise
525 Performance: A Systematic Review and Meta-Analysis. *Int J
526 Sports Physiol Perform*. 2016;11(1):4-14.
527 doi:10.1123/ijsp.2015-0204.
- 528 13. Jeukendrup A, Saris WH, Brouns F, Kester AD. A new
529 validated endurance performance test. *Med Sci Sports Exerc*.
530 1996;28(2):266-270.

- 531 14. Sharma V, Cunniffe B, Verma AP, Cardinale M, Yellon D.
532 Characterization of acute ischemia-related physiological
533 responses associated with remote ischemic preconditioning: a
534 randomized controlled, crossover human study. *Physiol Rep*.
535 2014;2(11):e12200-e12200. doi:10.14814/phy2.12200.
- 536 15. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity
537 of four pain intensity rating scales: *Pain*. 2011;152(10):2399-
538 2404. doi:10.1016/j.pain.2011.07.005.
- 539 16. Perneger TV. What's wrong with Bonferroni adjustments.
540 *BMJ*. 1998;316(7139):1236-1238.
- 541 17. Batterham AM, Hopkins WG. Making meaningful inferences
542 about magnitudes. *Int J Sports Physiol Perform*.
543 2006;1(1):50-57.
- 544 18. Cohen J. *Statistical Power Analysis for the Behavioral*
545 *Sciences*. 2. ed., reprint. New York, NY: Psychology Press;
546 2009.
- 547 19. Hopkins WG, Marshall SW, Batterham AM, Hanin J.
548 Progressive statistics for studies in sports medicine and
549 exercise science. *Med Sci Sports Exerc*. 2009;41(1):3-13.
550 doi:10.1249/MSS.0b013e31818cb278.
- 551 20. Jean-St-Michel E, Manhiot C, Li J, et al. Remote
552 Preconditioning Improves Maximal Performance in Highly
553 Trained Athletes: *Med Sci Sports Exerc*. 2011;43(7):1280-
554 1286. doi:10.1249/MSS.0b013e318206845d.
- 555 21. Cruz RS de O, de Aguiar RA, Turnes T, Pereira KL, Caputo
556 F. Effects of ischemic preconditioning on maximal constant-
557 load cycling performance. *J Appl Physiol*. 2015;119(9):961-
558 967. doi:10.1152/jappphysiol.00498.2015.
- 559 22. Patterson SD, Bezodis NE, Glaister M, Pattison JR. The
560 Effect of Ischemic Preconditioning on Repeated Sprint
561 Cycling Performance: *Med Sci Sports Exerc*.
562 2015;47(8):1652-1658.
563 doi:10.1249/MSS.0000000000000576.
- 564 23. Kjeld T, Rasmussen MR, Jattu T, Nielsen HB, Secher NH.
565 Ischemic Preconditioning of One Forearm Enhances Static
566 and Dynamic Apnea: *Med Sci Sports Exerc*. 2014;46(1):151-
567 155. doi:10.1249/MSS.0b013e3182a4090a.

- 568 24. Paixão R, da Mota G, Marocolo M. Acute Effect of Ischemic
569 Preconditioning is Detrimental to Anaerobic Performance in
570 Cyclists. *Int J Sports Med.* 2014;35(11):912-915.
571 doi:10.1055/s-0034-1372628.
- 572 25. Ferreira TN, Sabino-Carvalho JL, Lopes TR, et al. Ischemic
573 Preconditioning and Repeated Sprint Swimming: A Placebo
574 and Nocebo Study. *Med Sci Sports Exerc.* May 2016:1.
575 doi:10.1249/MSS.0000000000000977.
- 576 26. Lisbôa FD, Turnes T, Cruz RSO, Raimundo JAG, Pereira GS,
577 Caputo F. The time dependence of the effect of ischemic
578 preconditioning on successive sprint swimming performance.
579 *J Sci Med Sport.* September 2016.
580 doi:10.1016/j.jsams.2016.09.008.
- 581 27. Hittinger EA, Maher JL, Nash MS, et al. Ischemic
582 preconditioning does not improve peak exercise capacity at
583 sea level or simulated high altitude in trained male cyclists.
584 *Appl Physiol Nutr Metab.* 2015;40(1):65-71.
585 doi:10.1139/apnm-2014-0080.
- 586 28. Johnsen J, Pryds K, Salman R, Løfgren B, Kristiansen SB,
587 Bøtker HE. The remote ischemic preconditioning algorithm:
588 effect of number of cycles, cycle duration and effector organ
589 mass on efficacy of protection. *Basic Res Cardiol.*
590 2016;111(2). doi:10.1007/s00395-016-0529-6.
- 591 29. Crisafulli A, Tangianu F, Tocco F, et al. Ischemic
592 preconditioning of the muscle improves maximal exercise
593 performance but not maximal oxygen uptake in humans. *J*
594 *Appl Physiol.* 2011;111(2):530-536.
595 doi:10.1152/jappphysiol.00266.2011.
- 596 30. Kido K, Suga T, Tanaka D, et al. Ischemic preconditioning
597 accelerates muscle deoxygenation dynamics and enhances
598 exercise endurance during the work-to-work test. *Physiol*
599 *Rep.* 2015;3(5):e12395-e12395. doi:10.14814/phy2.12395.
- 600 31. Cruz RS de O, de Aguiar RA, Turnes T, Salvador AF, Caputo
601 F. Effects of ischemic preconditioning on short-duration
602 cycling performance. *Appl Physiol Nutr Metab Physiol Appl*
603 *Nutr Metab.* 2016;41(8):825-831. doi:10.1139/apnm-2015-
604 0646.
- 605 32. McMahon NF, Leveritt MD, Pavey TG. The Effect of Dietary
606 Nitrate Supplementation on Endurance Exercise Performance
607 in Healthy Adults: A Systematic Review and Meta-Analysis.

608 *Sports Med.* September 2016. doi:10.1007/s40279-016-0617-
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Figures:

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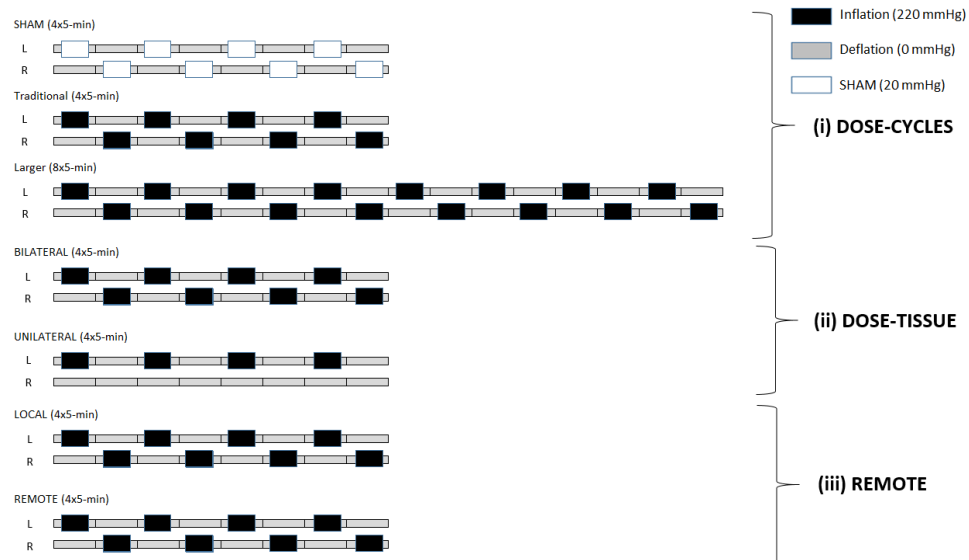
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629 Figure 1 – Schematic of different of IPC protocols (i) comparison of
 630 dose-cycles (ii) comparison of dose-tissue and (ii) comparison
 631 remote. (N.B. traditional dose of IPC was performed once in the
 632 experimental design but is shown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

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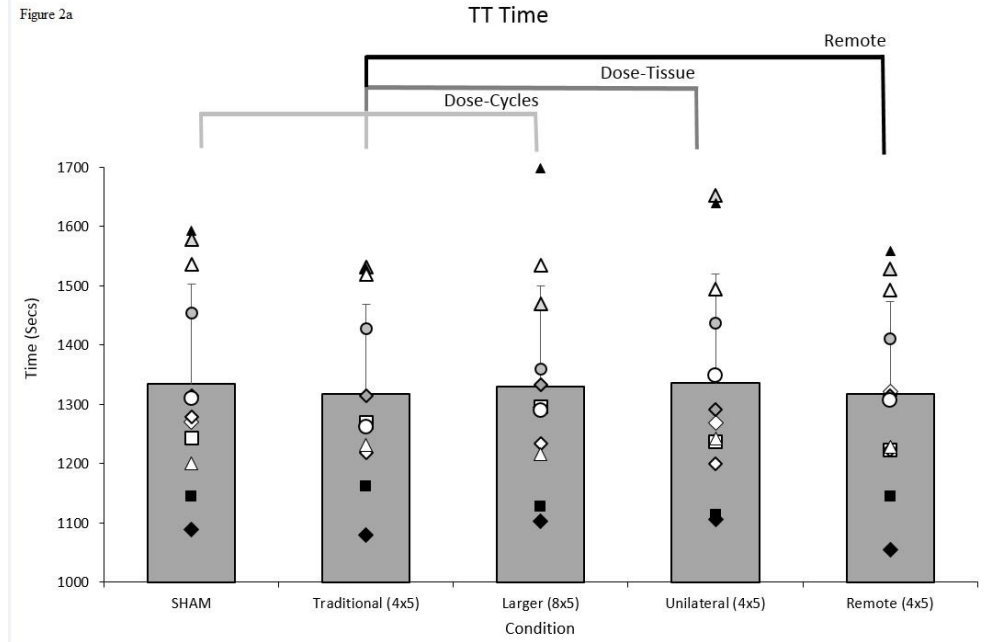
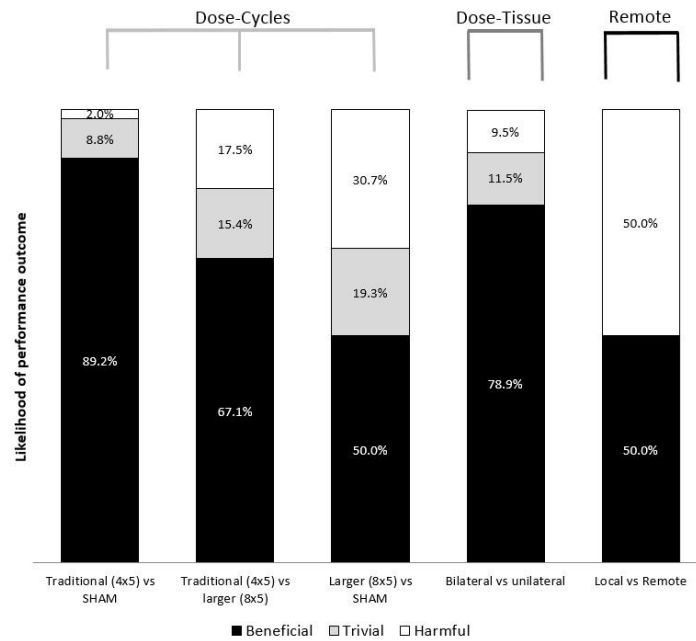


Figure 2b



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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.

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Tables:

651 Table 1: The effect of “dose-cycles” on power, heart rate, rate of
 652 perceived exertion and $\dot{V}O_2$ following 25%, 50%, 75% and 100%
 653 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
$\dot{V}O_2$ (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

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659 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%
 661 time points during time trial performance.

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	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
$\dot{V}O_2$ (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

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

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	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
$\dot{V}O_2$ (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

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

	Perceived discomfort of condition (ratings 0-10)					<i>Mean discomfort rating</i>
	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

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