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1 **IS DELAYED ISCHEMIC PRECONDITIONING AS EFFECTIVE ON**
2 **RUNNING PERFORMANCE DURING A 5-KM TIME TRIAL AS**
3 **ACUTE IPC?**

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20 **ABSTRACT**

21 Ischemic preconditioning (IPC) may enhance exercise performance. Cardioprotective effects of IPC
22 are known to re-occur 24-h after the stimulus. Whether the delayed effect of IPC has similar effects as
23 IPC on exercise performance is unknown.

24 **Objectives:** Examine whether IPC applied *24-hours* (24-IPC) before exercise is equally effective as
25 IPC in improving exercise performance.

26 **Design:** Randomized, cross-over study

27 **Methods:** 12 healthy participants were randomly exposed to SHAM-session, IPC or 24-IPC before a
28 self-paced 5 kilometer running trial on a treadmill. Subjects were blinded for time, speed and heart
29 rate. Furthermore, heart rate, BORG, and the local tissue saturation index were measured during
30 exercise, while lactate levels were determined after running. Using a regression model, we explored
31 whether these parameters predicted the change in running time after IPC and 24-IPC.

32 **Results:** We found no differences in finish time after IPC (SHAM: 1400 ± 105 seconds, IPC: $1381 \pm$
33 112 seconds, 24-IPC: 1385 ± 113 seconds; $P=0.30$). However, we observed a significant positive
34 relation between the change in finish time after IPC and 24-IPC ($P=0.016$; $r=0.677$). Using stepwise
35 linear regression, a lower post-exercise blood lactate level after IPC or 24-IPC was significantly
36 related to an improvement in finish time ($R^2=0.47$, $\beta=-0.687$, $P=0.007$).

37 **Conclusions:** Although no significant effect of IPC or 24-IPC on exercise performance was found,
38 individual finish time after IPC and 24-IPC were strongly correlated. Therefore, our data suggest that,
39 at the individual level, the effects of 24-IPC are closely related to the effects of IPC.

40 **KEYWORDS:** exercise performance; time trial; athletic performance; preconditioning; ischaemic
41 preconditioning;

42 **INTRODUCTION**

43 Ischemic preconditioning (IPC) was originally described as an effective strategy to protect cardiac
44 cells against a prolonged period of ischemia. In 1986, it was demonstrated that repeated bouts of local
45 ischemia caused delayed lethal cardiac muscle damage after a prolonged period of ischemia ¹. Most
46 studies have focused on the potential cardioprotective abilities, leading to large, multi-centre trials that
47 established the potency of IPC to attenuate cardiac damage and improve clinical prognosis ²⁻⁴.

48
49 De Groot *et al.* were the first to explore the ability of IPC to enhance physical performance ⁵. They
50 found significant improvement in exercise performance when a maximal cycle test was preceded by
51 IPC. Similar findings of performance enhancement were reported by others, ⁶⁻¹¹ but not all ¹¹⁻¹³. Those
52 studies were mainly designed to assess the direct effect of IPC on exercise performance, while the
53 working mechanism of IPC on skeletal muscle oxygenation status is less explored. The limited
54 number of studies that explored the working mechanism of IPC suggest that IPC may induce systemic
55 changes in blood flow through a change in sympathetic activity, whereas local changes in the muscle
56 (e.g. increase oxygen uptake or change in mitochondrial activity) are also likely to contribute to an
57 increase in muscle oxygenation {Horiuchi, 2015 #32;Kjeld, 2014 #15;Saito, 2004 #14}.

58
59 All previous studies exploring the impact of IPC to exercise performance timed IPC in close proximity
60 to the exercise event. This poses, however, practical limitations as IPC cannot always be applied in
61 close proximity to the start of an athletic event. Interestingly, in 1993, two independent groups
62 reported that the protective effect of IPC (which disappears within hours) reappears after
63 approximately 24 hours and can last up to 72 hours ^{14, 15}, commonly referred to as the second window
64 of protection (SWOP). Similar to the traditional IPC studies, the SWOP is associated with a significant
65 reduction in myocardial infarct size ¹⁵. These observations raise the question whether enhanced
66 performance is also present when IPC is applied 24-72 h prior to the exercise event to match the event
67 with SWOP. Practically, such timing would be preferred over the application of IPC immediately
68 before an athletic event.

69

70 The primary aim of this study, therefore, was to assess our hypothesis whether IPC applied *24-hours*
71 before the running trial (i.e. timed together with the start of the SWOP) is equally effective in
72 changing exercise performance compared to the application of IPC immediately before a running
73 event in healthy volunteers. Secondly, we explored whether the effect of (24-h) IPC is related to
74 changes in local tissue oxygenation (measured with Near-infrared Spectroscopy (NIRS)) of the vastus
75 lateralis muscle during running exercise and/or production of lactate at the end of exercise. Such
76 insight may help to better understand the potential mechanisms contributing to the exercise benefits of
77 (24-h) IPC in humans.

78

79

80 **METHODS**

81 Adopting a randomized, cross-over study, 12 healthy participants volunteered to participate. Baseline
82 characteristics are shown in Table 1. Subjects were moderate to well-trained amateur runners (Table
83 1), who exercised at least two hours a week, including a minimum of one hour running at moderate-to-
84 high intensity. We excluded older participants (>50 years), subjects with cardiovascular disease or any
85 other chronic disease effecting maximal performance as this may affect the efficacy of IPC ¹⁶. Prior to
86 participation, subjects were informed about the procedures of the study, but not about the rationale of
87 the study to keep subjects naive about the potential effect of IPC as well as the timing of IPC. All
88 subjects gave their written informed consent prior to participation. This study was approved by the
89 local ethics committee of the Radboud University Medical Centre.

90

91 Subjects visited our laboratory at six different occasions (including 3 familiarization sessions to
92 customise to the 5-km time trial), to perform a 5-km time trial on a treadmill. On the first day,
93 participants were examined prior to testing by a physician, comprising an assessment of an
94 electrocardiography under resting conditions. On all testing days, subjects refrained from alcohol,
95 caffeine, tea, chocolate and (intensive) physical exercise for at least 24 h prior to testing as these

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96 factors may potentially influence exercise performance. Additionally, subjects were asked to adopt a
97 similar eating pattern at all days of the measurements. Participants were tested at the same time of day
98 to control for diurnal variation and its impact on exercise performance ¹⁷, while measurements were
99 performed in a temperature-controlled testing room with the temperature set at 19°C. Testing days
100 were separated by at least 4 days, in order to prevent possible carry-over effects of the exercise bout
101 and/or IPC.

102 First, participants performed three familiarization sessions on a treadmill. Participants were instructed
103 to run a 5-km time trial on a motorized treadmill (EN-BO Systems, Bonte BV, the Netherlands) as fast
104 as possible, while being blinded for time, speed and heart rate, but not running distance. The
105 familiarization sessions indicated that the coefficient of variation for a self-paced 5-km time trial
106 varied between 1.6-1.8%. When being familiarized with running the 5-km trial, the experiment started.
107 In a randomized order, participants received IPC, 24-IPC and SHAM. Participants were informed that
108 all interventions, including SHAM, could potentially lead to an improved running performance to keep
109 them naive. After the application of IPC, participants performed a standardized warm-up, followed by
110 the 5-km time trial.

111

112 IPC was performed in the supine position using bilateral arterial occlusion ⁵. Occlusion cuffs were
113 positioned proximally around the thigh (bilaterally) and inflated to 220 mmHg to block arterial inflow
114 for 5 minutes, followed by a 5 minute deflation. This procedure was repeated 4 times, with each
115 ischemic episode separated by 5 minutes rest. For the IPC intervention, this procedure was started 1
116 hour before the time trial, whilst the procedure was timed exactly 24 hour prior to the time trial for the
117 24-IPC.

118 The control intervention was performed under the same conditions as the intervention test, but this
119 time the cuff was inflated to only 20 mmHg, which did not alter the arterial inflow. **No additional
120 control test (i.e. without cuff inflation) was performed to keep participants naïve regarding any
121 possible effects of the intervention and, subsequently, to prevent the possibility of a placebo effect.**

122

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123 After the IPC/SHAM-intervention, subjects were seated for 5 minutes. Subsequently, subjects
124 performed a 5-minute warm-up followed by another 5 minutes to stretch their muscles as preferred,
125 after which the 5-km running time trial was started. The 5-km running trial was performed on a
126 motorized treadmill. Main outcome parameter was finish time (Fisher Scientific, the Netherlands). The
127 speed of the treadmill was set at 11 km/h, where after participants were allowed to alter running speed,
128 but were kept blinded for running speed and running time. Participants were instructed to run 5-km as
129 fast as possible. The only information available to the participants during each time trial was total
130 distance covered (m) as to adjust work-output to pace towards the known endpoint. No further
131 information/feedback and/or encouragements were provided during the 5-km trial.

132

133 NIRS is based on the relative transparency of tissue to light in the near-IR region, and on the oxygen-
134 dependent absorption changes of hemoglobin (Hb) and myoglobin (Mb). Using a continuous-wave
135 near-IR spectrophotometer (Portamon, Artinis Medical Systems, BV, The Netherlands) that generates
136 light at 905, 850 and 770 nm, it is possible to differentiate between oxy- and
137 deoxyhemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb , respectively). NIRS measurements were
138 carried out on the belly of the vastus lateralis muscle 12 cm above the fibular head. Quantitative NIRS
139 measurements of muscle oxygenation (mVO_2) from O_2Hb were obtained during exercise and
140 subsequently, tissue saturation index (TSI) was calculated as the percentage of O_2Hb/O_2Mb of total Hb
141 and Mb.

142

143 Heart rate was measured continuously by a Polar chest band (Polar® RS 800) and recorded every
144 500m. Blood pressure was measured at the right arm before, during and after the IPC/SHAM-
145 intervention and after the time trial. Furthermore, a finger capillary blood sample (Accutrend® Lactate,
146 Roche Diagnostics, Mannheim, Germany) was taken before and after the IPC-/SHAM-intervention,
147 but also after the 5-km time trial (± 2 min after the exercise bout) to measure blood lactate levels. In
148 addition, the rate of perceived exertion was registered on a Borg-scale (6-20) during warm up, every
149 500 m and after the 5-km time trial.

150

151 Data is presented as mean \pm SD, unless stated otherwise. To examine differences in finish time
152 between the interventions, one-way repeated measures ANOVA was used. To examine whether IPC
153 and 24-IPC lead to comparable changes in exercise performance (i.e. primary aim of the study), we
154 used a Pearson correlation coefficient to relate changes in finish time after IPC *versus* 24-IPC. Using a
155 2-way ANOVA, we examined whether changes in TSI, heart rate, pace time and BORG during the
156 time trial (every 500m; 'time') differs across the 3 conditions ('intervention'; SHAM *vs* IPC *vs* 24-
157 IPC). Differences across the conditions were analyzed by repeated measures ANOVA. Finally, to
158 investigate which parameters contributed to the *change* in exercise performance after IPC or 24-IPC
159 (i.e. Δ IPC-control, Δ 24-IPC-control), a stepwise linear regression analysis was performed. Study
160 parameters included in this model were presented as the *change* between IPC versus control or 24-IPC
161 *versus* control. Differences were considered to be statistically significant at $P < 0.05$.

162

163

164 **RESULTS**

165 We found no differences in finish time between IPC, 24-IPC and SHAM ($P=0.30$, Figure 1A). Also no
166 significant changes were observed when calculating the change in finish time between IPC *versus*
167 SHAM (-16 ± 39 s, $P=0.14$) or 24-IPC *versus* SHAM (-16 ± 58 s, $P=0.29$). However, when pooling the
168 individual changes in finish time after IPC and 24-IPC, the majority of subjects (7 of 12) seem to have
169 an improved finish time, however this failed to reach statistical significance ($P=0.10$, Figure 1B,
170 Figure 2). Interestingly, we found a significant positive relation between the change in finish time after
171 IPC *versus* the change after 24-IPC ($P=0.016$; $r=0.677$, Figure 2). This relation indicates that the
172 change in finish time after IPC is strongly related to the change in finish time after 24-h IPC within
173 individuals.

174

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175 Heart rate gradually increased during the 5-km time trial, with no differences between the
176 interventions ($P=0.63$). Pace slightly decreased during the 5-km time trial in the three conditions, with
177 a significant time*treatment effect for 24-IPC ($P=0.016$). Post-hoc analysis showed that subjects
178 started at a higher pace during the 24-IPC time trial and ended with a somewhat lower pace compared
179 to the other two conditions. All participants reached a similar level of exertion at the end of the 3 time
180 trials (SHAM: 19 ± 2 BORG, IPC: 19 ± 2 BORG, 24-IPC: 19 ± 2 BORG; $P=0.60$). Blood lactate
181 levels significantly increased after the 5-km time trial, whilst no differences were found among the 3
182 trials in post-exercise lactate levels (SHAM: 6.4 ± 3.1 mmol/l, IPC: 8.0 ± 2.7 mmol/l, 24-IPC: $7.5 \pm$
183 2.2 mmol/l; $P=0.30$, $P=0.24$). Finally, TSI, as measured with NIRS on the vastus lateralis muscle,
184 showed a rapid decrease in saturation upon the start of the 5-km time trial and remained stable
185 thereafter. This time-dependent change was similar among all three conditions ($P=0.14$)

186

187 Stepwise linear regression was performed to identify whether exercise characteristics could predict the
188 change in finish time after IPC or 24-IPC. Post-exercise blood lactate concentration significantly
189 contributed to the change in running time after IPC or 24-IPC ($R^2=0.47$, $\beta=-0.687$, $P=0.007$). More
190 specifically, lower post-exercise blood lactate levels after IPC or 24-IPC were significantly related to
191 an improvement in finish time. The number of training hours and differences between trials in
192 maximal heart rate and TSI did not contribute to changes in finish time.

193

194 **DISCUSSION**

195 This is the first study to compare the impact of the application of IPC 24-hours before the exercise
196 bout *versus* IPC immediately before exercise on performance. Overall, we found no significant effect
197 of 24-IPC or IPC on exercise performance during a 5-km time trial in moderately-to-well trained
198 athletes. However, we found a strong and positive relationship between the change in finish time when
199 the 5-km trial was preceded by IPC *versus* 24-IPC. This suggests that, at an individual level, 24-IPC
200 exerts a comparable effect on exercise performance compared to IPC during a 5-km running time trial.

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201 Practically, athletes competing at a high level could apply IPC 24h before their sporting event.
202 Finally, improvement in finish time was strongly related to a decrease in post-exercise blood lactate
203 levels, but not to changes in vastus lateralis muscle oxygenation. Taken together, at an individual
204 level, the effect sizes of 24-IPC and IPC during a 5-km time trial are strongly related.

205

206 We did not find a significant effect of IPC on exercise performance, which contrast with some ⁵⁻¹¹, but
207 not all studies ¹¹⁻¹³. Although not significant, the effect size found in our study is 1.4% reduction in
208 running time, which corresponds well with other studies that reported a 1- 4% improvement in peak
209 workload ^{5, 8}, improvement in finish time ^{6, 7, 10} or prolonged time to exhaustion ^{9, 11}. One potential
210 explanation for the absence of a significant effect is that our self-selected exercise protocol resulted in
211 a relatively large variation in running time within participants despite three familiarization sessions
212 before the actual start. These types of exercise trials are importantly influenced by the athlete's
213 experience and motivation ¹⁸⁻²¹. As a result, the coefficient of variation of the self-paced 5-km time
214 trial (1.6-1.8%) is somewhat higher than *a priori* expected. Consequently, more subjects may be
215 necessary to detect differences when adopting the self-paced 5-km time trial. In support of this
216 hypothesis, previous work that reported an effect of IPC to improve exercise performance adopted an
217 exercise protocol where subjects performed exercise at maximal effort level. Indeed, these previous
218 studies adopted exercise tests that caused substantially larger blood lactate concentration (~12-13
219 mmol/l) ^{5, 7} compared to our protocol (~6-8 mmol/l). Taken together, although we found no significant
220 effect of IPC on exercise performance, the effect size of IPC is comparable to previous studies in this
221 field.

222

223 The primary aim of our study was to explore whether 24-IPC and IPC would cause comparable
224 changes in running time. In agreement with our hypothesis, a strong and positive correlation was
225 found between the change in running time after IPC and 24-IPC. Similarly, those who did not show
226 improvement in running time after IPC, also showed no change after 24-IPC. Although our study is
227 the first in the literature to demonstrate that IPC and 24-IPC have comparable effects on exercise

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228 performance, these findings are largely in agreement with previous work on IPC and protection of
229 cardiac damage by SWOP. Previous work on the cardioprotective effects of IPC and 24-IPC found
230 comparable protection against prolonged ischaemia ²²⁻²⁴, although some data is conflicting ²⁵. Taken
231 together, our data extends previous work in the area of cardiology, in that the effect of 24-IPC on
232 exercise performance is comparable to that observed after IPC.

233

234 In an attempt to better understand the underlying mechanisms, we explored tissue saturation of the
235 vastus lateralis muscle during the time trial. Previous work in animals linked IPC to improved muscle
236 oxygenation during exercise ²⁶. Studies that have measured TSI, report a 20% decrease in TSI after
237 IPC relative to control ^{10, 27}. In our study, we found that IPC and 24-IPC exerted a similar impact on
238 TSI during exercise than SHAM ²⁷. This observation suggests that IPC and 24-IPC did not alter
239 muscle oxygen delivery during exercise ²⁸. This is supported by our regression analysis.

240

241 An alternative explanation for the underlying mechanisms relates to blood lactate levels during
242 exercise. Previous work demonstrated that changes in blood lactate concentration and mitochondrial
243 capacity account for 68% of the variation in cycling time trial performance ²⁹. Furthermore, lower
244 blood lactate concentrations at a given workload improves endurance exercise in various populations,
245 including in highly trained ²⁴. These observations are somewhat in line with earlier observations from
246 Bailey *et al.*, who demonstrated that IPC lowers blood lactate levels during running exercise at
247 submaximal intensity ⁶. Interestingly, running speed associated with lower blood lactate levels after
248 IPC matched with the running speed of the 5-km time trial ⁶. Therefore, our findings provide some
249 further support that IPC may enhance exercise performance through changes in the lactate pathways.

250 Although we included three familiarizations sessions prior to testing, the relatively high day-to-day
251 variability of 1.6-1.8% in athlete's performance could have influenced our results, especially since the
252 effect size of 1.4 % was somewhat smaller than initially anticipated. Interestingly, when all data from
253 IPC and 24-IPC are pooled (n=24), we found a trend for a decline in the time trial ($P=0.10$).

254 *Conclusions*

- 255 • A strong relation is present between acute IPC and 24-IPC, which suggests that the effects of
256 IPC and 24-IPC are closely related on an individual level.
- 257 • Lower post-exercise blood lactate levels after IPC or 24-IPC is significantly related to an
258 improvement in finish time.
- 259 • Differences in TSI, did not contribute to changes in finish time.

260 *Practical Implications*

- 261 • IPC may improve exercise performance, although further research is necessary to indicate
262 whether exercise intensity, and hence blood lactate levels are important determinants for an
263 IPC effect.
- 264 • On an individual level, 24-IPC is as effective as acute IPC, which implicates that both can be
265 used preceding a contest.
- 266 • 24-IPC m represents a more feasible and practical approach compared to the application of
267 IPC immediately before the exercise event.

268

269

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273

274

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347

348

349

350 **FIGURE LEGENDS**

351 **FIGURE 1.** Individual presentation (n=12) of the finish time during the SHAM, IPC and 24-IPC
352 intervention (A, in seconds) and the change in finish time compared to SHAM after
353 application of IPC or 24-IPC (B, in seconds). A negative value in B relates to a better
354 finish time. Each dot represents a single participant. The horizontal line represents the
355 average, with the error bars representing the SE.

356
357 **FIGURE 2:** Correlation between the individual changes in finish time compared to SHAM after
358 application of IPC (X-axis, in seconds) and the change in finish time between SHAM
359 and 24-IPC (Y-axis, in seconds) in our participants (n=12). A negative value on both
360 axes relates to a better finish time after IPC or 24-IPC. The dotted line represents the
361 regression line from the Pearson's correlation coefficient.