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Is delayed ischemic preconditioning as effective on running performance during a 5km time trial as acute IPC?

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

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ABSTRACT

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

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

Design: Randomized, cross-over study

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

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

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

KEYWORDS: exercise performance; time trial; athletic performance; preconditioning; ischaemic preconditioning;

INTRODUCTION

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

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

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

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

METHODS

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

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

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 19ºC. 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

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.

Data is presented as mean ± SD, unless stated otherwise. To examine differences in finish time between the interventions, one-way repeated measures ANOVA was used. To examine whether IPC and 24-IPC lead to comparable changes in exercise performance (i.e. primary aim of the study), we used a Pearson correlation coefficient to relate changes in finish time after IPC *versus* 24-IPC. Using a 2-way ANOVA, we examined whether changes in TSI, heart rate, pace time and BORG during the time trial (every 500m; 'time') differs across the 3 conditions ('intervention'; SHAM *vs* IPC *vs* 24-IPC). Differences across the conditions were analyzed by repeated measures ANOVA. Finally, to investigate which parameters contributed to the *change* in exercise performance after IPC or 24-IPC (i.e. ΔIPC-control, Δ24-IPC–control), a stepwise linear regression analysis was performed. Study parameters included in this model were presented as the *change* between IPC versus control or 24-IPC *versus* control. Differences were considered to be statistically significant at $P<0.05$.

RESULTS

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

Conclusions

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

Practical Implications

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

Acknowledgments

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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.