

International Journal of Research in Exercise Physiology

Original Research Article

The Effects of Delayed Ischemic Preconditioning on Performance and Recovery in Repeated Cycling Bouts

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ABSTRACT

Introduction: Ischemic preconditioning (IPC) is a technique of systematically occluding and reperfusing blood vessels that has shown to induce a biochemical cascade that increases the availability of nitric oxide (NO) and adenosine and enhances blood flow, endothelial function, and ATP regeneration. IPC has two physiological windows of protection; the first (immediate) window take place within the first 12 hours of treatment, while the second (delayed) takes place 24-72 hours post-treatment. The aim of this study was to evaluate its effects cyclists performing repeated incremental cycling bouts. **Methods:** In a randomized, crossover study, subjects received a 4 x 5min bilateral IPC treatment to the thigh. 24 and 48 hours after treatment, they performed an incremental cycling trial, followed by a 15-min recovery period, and then repeated the same cycling trial and recovery period. Time to fatigue (TTF) was recorded for each trial and heart rate (HR), blood lactate (La^-), and blood **Results:** pressure (BP) were recorded throughout the recovery period. Despite individual variability, delayed IPC improved TTF ($p<0.05$), decreased performance decrement between trials, lowered HR and BP ($p<0.05$), which allowed cyclists to work at higher lactate levels ($p<0.05$). **Conclusions:** These results suggest that delayed IPC may be an effective performance and recovery strategy for endurance athletes.

KEYWORDS: Ergogenic aid, cycling performance, lactate, fatigue, recovery

Introduction

The popularity of endurance events, and in particular, multi-day events, continues to increase. With the growing popularity of events such as the Tour de California, Ride the Rockies, along with multi-day destination cycling trips, it is important for athletes to focus not only on their

performance, but also their recovery in order to complete these multi-day events. Such events are particularly challenging as athletes need not only have the ability to recover between multiple climbs and sprints during each stage, they must also be able to recover from each day's challenges to perform well in the subsequent stage. In short, during these longer events, the ability

to recover has a large impact on an athlete's ability to perform.

During a day of competitive cycling, athletes need to perform at high and medium intensities, with short bouts at lower intensities to recover and usually, refuel. Adenosine Triphosphate (ATP) is the body's energy molecule and must be present for muscles to contract and for any degree of force to be exerted. ATP must also be continuously regenerated to allow work to continue. All three energy systems will be called into action during an endurance cycling event; the phosphagen system during short sprints at an all-out effort (maximum 10-12 seconds), the glycolytic system during high and medium-intensity, long-duration climbs, and oxidative system during lower-intensity, long-duration efforts¹. When the glycolytic system is in action, ATP is produced by breaking down carbohydrates (CHO) from dietary glucose or from stored glycogen in the muscles. Aside from ATP, pyruvate and NADH+H⁺ are produced. At higher intensities, pyruvate can further be converted to lactate by combining with NADH and H⁺. Lactate can act as a buffer or as a fuel source when it is oxidized and releases NAD⁺ to sustain glycolysis². However, there comes a point when the demand for ATP exceeds the body's ability to remove H⁺ and as they begin to accumulate, this leads to a decrease in pH and, eventually, to metabolic acidosis. In a state of metabolic acidosis, ATP production and enzyme action are inhibited, as are troponin and tropomyosin, because these protons

compete with Ca²⁺ binding to interfere with muscle contraction, and motor recruitment and motor pathways are impaired. Hyperventilation may also occur to help buffer the change in pH, however this comes with a greater O₂ cost that can compete with the O₂ available to the exercising skeletal muscle.

At higher intensities, lactate is formed as a result of accumulated protons and can actually act as a buffer to protect from metabolic acidosis. The body's ability to sustain a high level of intensity exercise depends largely on the body's ability to buffer or consume protons in order to maintain pH balance and stave off metabolic acidosis^{2,3,4}. Thus, the concurrent presence of lactate and acidosis should be a positive sign that the body is working hard to maintain a sustainable pH level and can also indicate that the body is in a state of high glycolytic flux⁵.

A number of studies have shown that an increase in muscular blood flow assists with the efflux of H⁺ and lactate and therefore that exercise helps with the removal of these energy byproducts^{3,6,7}. One method that has shown promise in increasing blood flow is a treatment known as Ischemic Preconditioning, or IPC.

IPC is a technique of systematically occluding and reperfusing blood vessels with a blood pressure cuff^{8,9}. IPC was first used clinically as a means of protecting vital organs from reperfusion injury and preventing

cumulative metabolic deficits and myocardial ischemia¹⁰ during certain types of surgical procedures.

IPC has two physiological windows of protection. The first window, known as the immediate response, takes place during the first 12 hours of treatment. The second window, or delayed response, takes place 24-72 hours post treatment^{11,12}.

IPC has been shown to induce a biochemical cascade that increases the availability of nitric oxide (NO) and adenosine, and enhances blood flow, endothelial function, and ATP regeneration^{13,14}. This naturally led exercise physiologists to explore whether the benefits from this biochemical cascade could be harnessed to enhance athletic performance.

Several studies performed on cyclists found that IPC does indeed appear to have an ergogenic effect by increasing time to fatigue, improving endothelial function; increasing O₂ consumption, stroke volume, and substrate delivery; improving the clearance of metabolites^{15,16,17} as well as an improvement in cycling performance^{17,18,19}. Although researchers are not entirely certain of the exact mechanisms at play, Salvador et al., (2016a) determined through an extensive meta-analysis that the beneficial effect of IPC was observed mainly during times of high metabolic demand, whereas sprint and power performance did not seem to be influenced. Their analysis suggests that results are dependent on aerobic pathways

and perhaps also an increase in central motor drive. The central effects of fatigue locomotor muscles can lead to an inhibition of central motor drive, and this feedback can place limitations on endurance efforts²¹.

It should be noted that most studies related to IPC and athletic performance have been immediately following treatment and there is very little research examining the delayed effects of IPC on performance^{11,12,22}. There also have been no published studies to date examining the effects on IPC on lactate recovery kinetics in multiple exercise bouts. Much of the literature supports that IPC does enhance submaximal and maximal aerobic performance, although the delayed response of IPC on performance and recovery has not yet been determined.

Despite the lack of specific research on IPC and lactate recovery kinetics, there is a clear connection between lactate and H⁺ consumption and blood flow dynamics with respect to metabolic acidosis. It was hypothesized that since IPC improves blood flow and tissue oxygenation, it would have a positive effect not only on performance and time to fatigue, but would have also a positive effect on subsequent recovery.

Methods

Participants

Ten healthy, recreational, non-elite cyclists (5 males and 5 females, characteristics described in Table 1 below) volunteered to participate in this randomized, double-blind, crossover study.

Table 1. Descriptive Characteristics of Participants, Mean \pm SD

| Baseline Parameter | Males (n=5) | Females (n=5) |
|---------------------|-------------------|-------------------|
| Age (years) | 37.6 \pm 3.0 | 36.6 \pm 3.9 |
| Height (cm) | 174.0 \pm 9.9 | 169.4 \pm 8.1 |
| Weight (kg) | 76.1 \pm 6.2 | 66.9 \pm 13.2 |
| BMI | 25.1 \pm 1.1 | 23.2 \pm 2.8 |
| Systolic BP (mmHg) | 114.4 \pm 6.1 | 109.4 \pm 8.8 |
| Diastolic BP mmHg) | 70.8 \pm 6.9 | 72.0 \pm 6.0 |
| Total Cholesterol | 204.2 \pm 22.48 | 193.6 \pm 47.01 |
| LDL Cholesterol | 114.2 \pm 12.6 | 95.2 \pm 31.0 |
| HDL Cholesterol | 69.2 \pm 13.2 | 84.8 \pm 18.0 |
| Triglycerides | 100.2 \pm 62.2 | 89.8 \pm 38.9 |
| Glucose | 90.6 \pm 6.1 | 86.8 \pm 3.3 |
| VO ₂ max | 45.6 \pm 5.5 | 36 \pm 5.2 |

Subjects were blinded to the effect of IPC on exercise performance and recovery and were not informed about the purpose of the study. Subjects were also screened for cardiovascular and metabolic risk factors, which can possibly influence the effectiveness of IPC treatment²³. All subjects were acclimatized to the elevation of the High Altitude Performance (HAP) Lab, located at 2350 meters. All subjects were fully informed of the study procedures and associated risks before completing informed consent, physical activity readiness (PAR-Q), and medical history questionnaires. The Institutional Review Board (IRB) at Western State Colorado University reviewed and approved this research project [HRC2017-02-03R53].

Experimental Design

Subjects reported to the HAP Lab for a total of eight visits. Visit 1 involved lipids and glucose testing in a fasted state and recording of anthropomorphic

measurements. Subjects who met the inclusion criteria returned to the HAP Lab at least 24 hours later for Visit 2 and completed a VO₂max test to calculate peak oxygen uptake and maximal watts (W). Visit 3 took place no earlier than seven days and consisted of a randomized IPC (IPC) or placebo IPC treatment (CON). Twenty-four hours later, subjects returned for Visit 4 and performed two incremental cycling tests followed by 15 minutes of active recovery. Capillary blood samples were taken by fingerprick at baseline and during the recovery period to analyze for blood pH and blood lactate concentrations. Blood pressure was taken in a seated position prior to and at completion of the testing session. Blood pressure was also taken between cycling bouts, at the end of the 15-minute recovery period, while subjects were seated on the bike. Visit 5 took place 24 hours later and the same incremental cycling test, including recovery bouts and blood analysis, was repeated. Visit 6 took place a minimum

of seven days after Visit 5 for males and four weeks later for females to control for menstrual cycle. This visit involved the opposite IPC treatment of Visit 3. Visits 7 and 8 took place 24 hours and 48 hours later,

respectively, and were replications of Visits 4 and 5. The lead researcher as well as study subjects were blinded to the IPC and CON treatments. Figure 1 provides an overview of the visits.

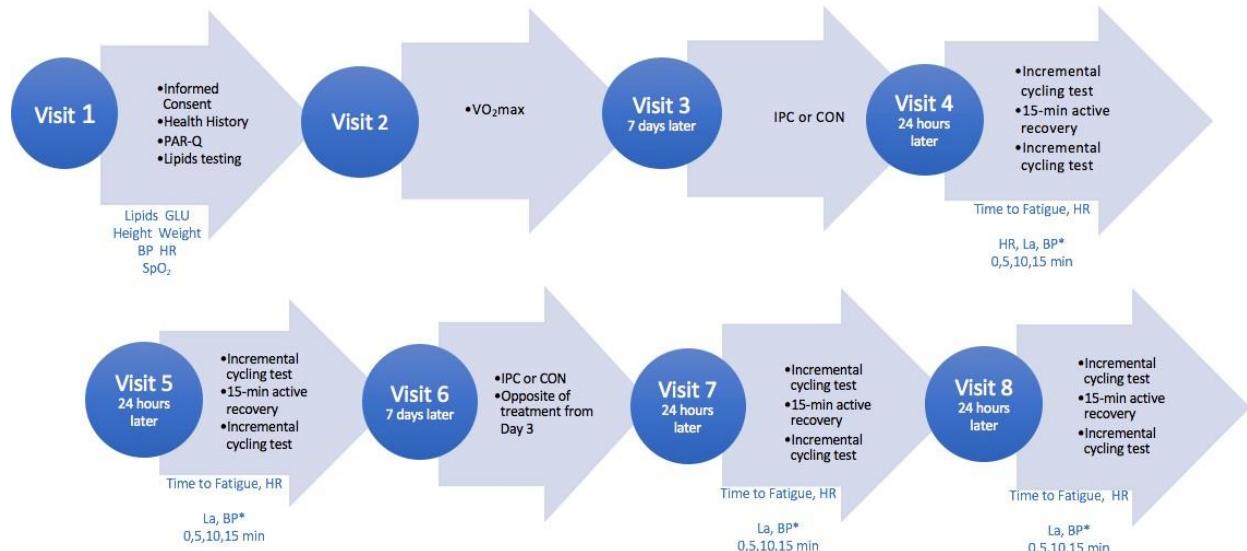


Figure 1. Experimental Flow Chart. GLU (glucose), BP (blood pressure), SpO₂ (blood oxygen saturation), VO₂max (maximal oxygen uptake), IPC (ischemic preconditioning treatment), CON (placebo treatment), RPE (rate of perceived exertion), HR (heart rate), La (blood lactate concentration). *BP (Blood pressure) was taken at the end of each recovery period.

Protocols

Height and Weight

Subjects' weights and heights were measured to the nearest 0.1kg and 0.5cm, respectively, using a medical grade scale and stadiometer (Tanita, Tokyo, Japan). Participants were instructed to wear light clothing and to remove shoes for the purpose of the measurements. BMI was also calculated to ensure healthy body composition. The equation used to calculate BMI was: $BMI = \text{kg}/\text{m}^2$.

Blood Pressure, Resting Heart Rate, and Blood-Oxygen Saturation

Participants were requested to sit

comfortably and quietly for a minimum of five minutes before measurements were taken. RHR and SpO₂ were measured using a Gurin Products Fingertip Pulse Oximeter (Tustin, CA). BP was measured using a sphygmomanometer (Medline, Mundelein, IL) around the brachial artery and a stethoscope for Korotkoff sounds. Three measurements were taken and averaged for validity. All resting measurements were taken by the primary investigator to ensure consistency.

Fasting Lipids and Blood Glucose

Subjects were instructed to fast for a minimum of 10 hours and not to consume

caffeine within this fasting period. Participants washed and warmed their hands once they arrived at the HAP Lab. An alcohol swab was used to clean the finger and dried with gauze before puncturing with a lancet. A fingerstick sample was collected with a heparin-coated 40 μ l capillary tube without milking the finger. The sample was immediately dispensed onto a test cassette for analysis in a Cholestech LDX System device (Alere, Inc., Waltham, MA). This device measured low-density lipoprotein (LDL) cholesterol, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, triglycerides (TG), and blood glucose (GLU). Studies have revealed that this system of analysis shows reliability with standard

clinical laboratory measurements of plasma proteins and lipoproteins²⁴.

Ischemic Preconditioning

The IPC treatment was performed in a supine position and the occlusion cuff was placed around the upper thigh and inflated to 220mmHg for five minutes. The cuff was removed and then immediately placed on the opposite thigh for 5 minutes of occlusion. This process was repeated a total of four times over a 40-minute period as shown in Figure 2. For the placebo treatment, the same protocol was followed, but the cuff was instead inflated to 40mmHg.

IPC and CON treatment

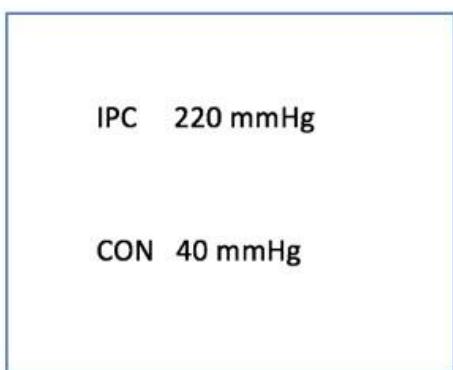
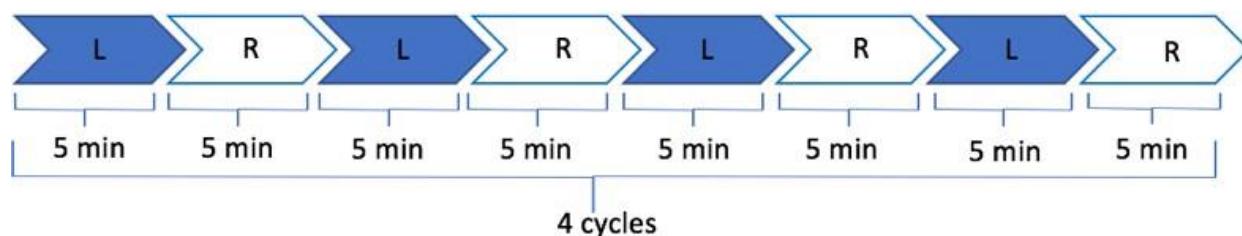


Figure 2. IPC treatment protocol. IPC (ischemic preconditioning treatment), CON (placebo treatment), L (left thigh), R (right thigh).

Recovery Measurements

Each incremental cycling test was followed by a 15-minute active recovery at 20% of maximal W. During each active recovery period, fingerprick blood samples were taken from fingertips at minutes 0, 5, 10, and 15. Blood was collected on a test strip and lactate concentration was measured using the Lactate Plus device (Nova Biomedical, MA). HR was recorded at these same time points. BP was also taken at the 15-minute mark. Resting, baseline measurements were also taken for blood lactate, HR, and BP in the same methods described above.

Statistical Analyses

Measurements were analyzed using the Statistical Package for Social Sciences (SPSS) Version 24.0 (IBM Corporation, Armonk, NY). All variables were initially checked for normality of variance using the Kolmogorov-Smirnov test. Measures of centrality and spread are presented as frequency and mean \pm standard deviation (SD) and Eta squared for effect size (ES). Two-way repeated measures ANOVA was used to compare time to fatigue between the IPC treatment and CON groups. Repeated measures ANOVA was used to compare the change in lactate kinetics, HR, and BP between the IPC treatment groups. The alpha level of statistical significance was set at $p < 0.05$ for all analyses.

Results

All IPC treatments and testing sessions were well tolerated by all subjects and 10 out of 10 subjects completed the full experiment. One of the female subjects experienced some bruising to her upper thighs 24 hours after receiving IPC treatment, but there was no discomfort reported from any other subject.

Performance- Time to Fatigue

There was a significant main effect of treatment on TTF ($p < 0.05$, ES 0.882), representing a large effect size. There was also a significant main effect of trial on TTF ($p < 0.05$, ES 0.354). IPC trials lasted an average of 21% longer than the Placebo trials (1A 13%, 1B 31%, 2A 23%, 2B 16%). The highest mean performance time was found in IPC Trial 2A, and 8 of the 10 subjects had a greater performance time when compared to their Placebo Trial 2A. There was also a lower performance decrement in the IPC trial vs. the Placebo trials between trials 1A and 1B (21% vs 28%), although statistical significance was only found between time points 2A and 2B in the IPC trials ($p < 0.05$, ES 0.645, see Table 2). Although the performance decrement was greater in the IPC trials between trials 2A and 2B, the greatest TTF was achieved during IPC Trial 2A, while IPC Trial B was on par with the TTF of Placebo Trial 1A.

Table 2. Performance time in seconds (mean \pm SD).

| | Trial 1A (n=10) | Trial 1B (n=10) | Change A to B | Trial 2A (n=10) | Trial 2B (n=10) | Change A to B |
|----------------------|--------------------------------|--------------------------------|------------------|--------------------|---------------------------------|-------------------------------|
| TTF Placebo (sec) | 174.1 \pm 71.8 | 126.0 \pm 53.2 | -49.2 \pm 69.5 | 162.8 \pm 75.6 | 136.8 \pm 61.7 | -21.4 \pm 34.6 |
| TTF IPC (sec) | 196.8 \pm 101.5 [†] | 164.2 \pm 69.3 ^{*†} | -32.6 \pm 58.1 | 200.7 \pm 86.8 | 158.4 \pm 68.7 ^{*†‡} | -42.3 \pm 33.1 [‡] |

Time to fatigue (TTF). Ischemic preconditioning (IPC). *Significant main effect of trial bout on TTF. † Significant main effect of treatment on TTF. ‡ Post-hoc testing revealed significant difference between time point and previous time point.

As demonstrated in Figure 3, performance times varied among individual subjects. In each of the trials, between six and eight individuals experienced a greater improvement in their TTF after receiving the IPC treatment. The IPC group demonstrated a mean performance time in Trial 2B that was on par with the Placebo group Trial 2A.

Overall, five individuals achieved greater TTF in all IPC trials, one individual achieved greater TTF in three IPC trials, two individuals had greater TTF in two of the IPC trials, and one individual improved during one of the IPC trials. There was only one individual who did not improve TTF during IPC trials.

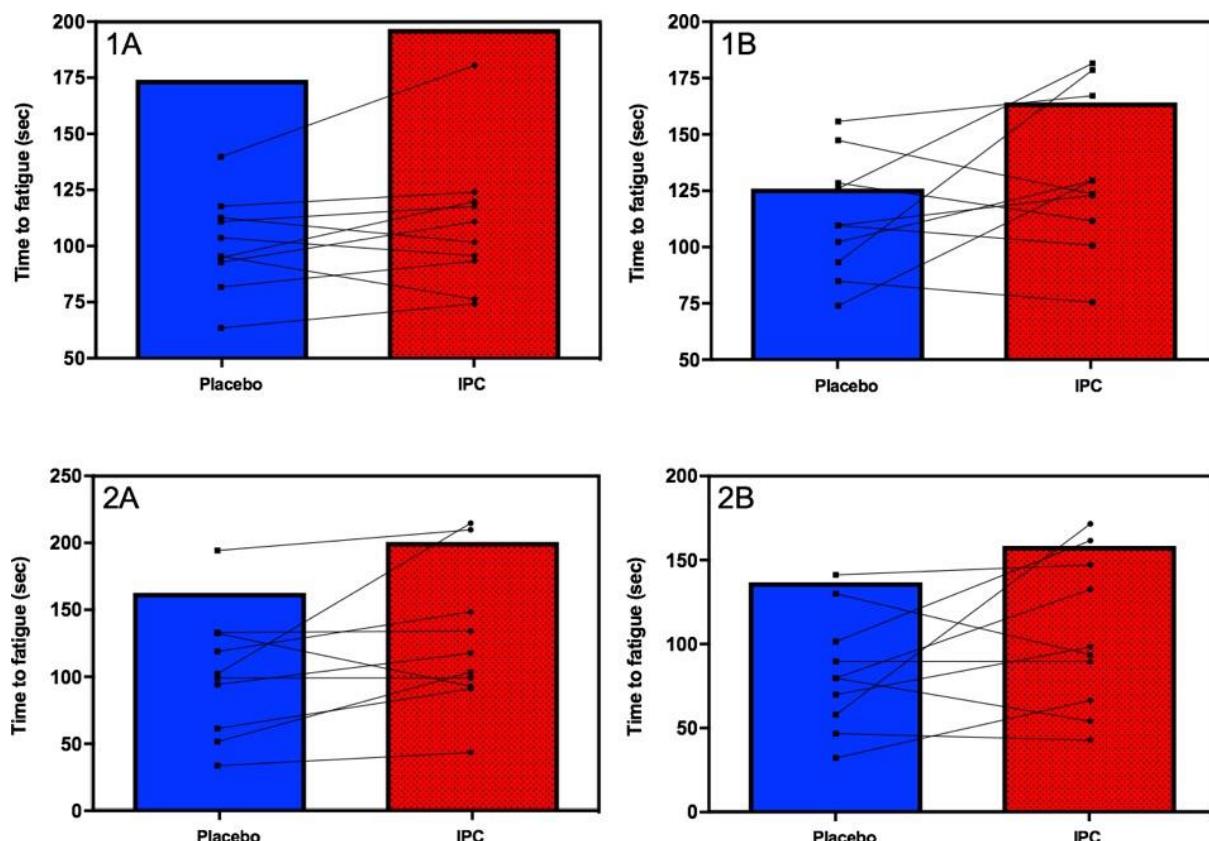


Figure 3. Performance time (TTF) mean responses for Placebo and IPC groups for each time trial (1A, 1B, 2A, and 2B). The Placebo and IPC trials are represented by solid and dotted bars, respectively. Individual TTF response are indicated by lines and circles and demonstrate whether the individual's performance increased, decreased, or showed no change. Mean responses show a significant change in performance with IPC

treatment, despite individual response.

Blood Lactate Recovery

A significant main effect of treatment ($p<0.05$) was found at all time points during all trials (ES 0.93, 0.877, 0.894, and 0.901 during trials 1A, 1B, 2A, and 2B, respectively). A significant effect main of time ($p<0.05$) was also found at all time

points during all trials (ES 0.678, 0.636, 0.781, and 0.797 during trials 1A, 1B, 2A, and 2B, respectively, see Table 3). Although not statistically significant, blood lactate was consistently higher in all IPC trials when compared to the Placebo trials and appeared to recover at a similar rate.

Table 3. Mean blood lactate measurements \pm SD for each recovery time point for all trials following placebo and IPC treatment.

| | 0 min | 5 min | 10 min | 15 min | %Change |
|-------------------------|----------------|-----------------------------|-----------------------------|-----------------------------|---------------|
| Placebo Trial 1A | 10.7 \pm 2.7 | 8.0 \pm 2.5 ^{*†} | 5.7 \pm 2.5 ^{*†} | 3.7 \pm 1.6 ^{*†} | -70 \pm 0.1 |
| IPC Trial 1A | 11.4 \pm 2.3 | 8.7 \pm 2.7 ^{*†} | 6.4 \pm 2.4 ^{*†} | 4.8 \pm 2.4 ^{*†} | -60 \pm 0.1 |
| Placebo Trial 1B | 9.7 \pm 2.9 | 6.9 \pm 3.3 ^{*†} | 5.2 \pm 3.1 ^{*†} | 3.6 \pm 2.3 ^{*†} | -60 \pm 0.1 |
| IPC Trial 1B | 10.4 \pm 3.0 | 8.3 \pm 3.6 ^{*†} | 6.2 \pm 2.8 ^{*†} | 5.0 \pm 2.7 [*] | -50 \pm 0.1 |
| Placebo Trial 2A | 9.5 \pm 2.2 | 7.2 \pm 2.3 ^{*†} | 4.9 \pm 2.4 ^{*†} | 3.4 \pm 2.0 ^{*†} | -70 \pm 0.1 |
| IPC Trial 2A | 10.7 \pm 2.7 | 8.2 \pm 3.1 ^{*†} | 5.7 \pm 2.6 ^{*†} | 4.3 \pm 3.0 ^{*†} | -60 \pm 0.1 |
| Placebo Trial 2B | 9.2 \pm 2.2 | 7.0 \pm 3.0 ^{*†} | 5.3 \pm 2.1 ^{*†} | 3.1 \pm 1.5 ^{*†} | -70 \pm 0.1 |
| IPC Trial 2B | 9.5 \pm 2.4 | 7.4 \pm 3.0 ^{*†} | 5.8 \pm 2.7 ^{*†} | 4.0 \pm 2.3 ^{*†} | -60 \pm 0.1 |

* Significant change from time point to immediately post-exercise. † Significant change from time point to previous time point.

Heart Rate Recovery

A significant main effect of treatment ($p<0.05$) was found at all time points during all trials (ES 0.994, 0.99, 0.995, and 0.995 during Trials 1A, 1B, 2A, and 2B, respectively. A significant main effect of time ($p<0.05$) was also found at all time points during all trials (ES 0.578, 0.600, 0.771, and 0.671 during

Trials 1A, 1B, 2A, and 2B, respectively, see Table 4). Although not statistically significant, HR and HR recovery rate were similar in both groups, despite the higher output observed during the IPC trials. The higher output was demonstrated by higher TTF and lactate levels.

Table 4. Mean HR measurements \pm SD for each recovery time point for all trials following placebo and IPC treatment.

| | 0 min | 5 min | 10 min | 15 min | %Change |
|-------------------------|-------------------|--------------------------------|-------------------------------|-------------------------------|----------------|
| Placebo Trial 1A | 146.1 \pm 13.0 | 119.7 \pm 13.4 ^{*†} | 118.8 \pm 12.7 [*] | 119.0 \pm 10.2 [*] | -18 \pm 0.9 |
| IPC Trial 1A | 146.8 \pm 15.7 | 120.4 \pm 12.4 ^{*†} | 121 \pm 11.6 [*] | 119.7 \pm 12.2 [*] | -18 \pm 0.1 |
| Placebo Trial 1B | 150.5 \pm 20.14 | 118.5 \pm 14.4 ^{*†} | 117.6 \pm 13.2 [*] | 117.3 \pm 11.1 [*] | -21 \pm 0.1 |
| IPC Trial 1B | 148.5 \pm 19.6 | 122.0 \pm 13.8 ^{*†} | 120.8 \pm 12.5 [*] | 121.2 \pm 12.2 [*] | -18 \pm 0.9 |
| Placebo Trial 2A | 142.9 \pm 10.0 | 115.9 \pm 9.5 ^{*†} | 116.0 \pm 9.3 [*] | 114.0 \pm 9.1 [*] | -20 \pm 0.8 |
| IPC Trial 2A | 145.6 \pm 17.2 | 120.3 \pm 11.3 ^{*†} | 119.2 \pm 9.8 [*] | 117.2 \pm 10.9 [*] | -19 \pm 0.1 |
| Placebo Trial 2B | 146.4 \pm 15.1 | 117.0 \pm 11.6 ^{*†} | 118.3 \pm 10.4 [*] | 117.1 \pm 11.7 [*] | -19 \pm 0.9 |
| IPC Trial 2B | 147.9 \pm 17.7 | 120.3 \pm 9.7 ^{*†} | 118.9 \pm 10.9 [*] | 119.5 \pm 10.9 [*] | -18 \pm 0.9 |

* Significant change from time point to immediately post-exercise. † Significant change from time point to previous time point.

Mean Arterial Pressure Change

A significant main effect of treatment ($p<0.05$) was found at all time points during all trials (ES 0.998, 0.999, 0.998, and 0.998 during trials 1A, 1B, 2A, and 2B, respectively). MAP only increased

significantly at one time point in the IPC group at the end of the recovery period. Although not statistically significant, MAP was lower at the start of all IPC trials when compared to the Placebo trials (see Table 5).

Table 5. Mean Arterial Pressure (MAP, mmHg) measurements \pm SD taken at the end of each recovery time point for all trials following placebo and IPC treatment.

| | Baseline | 15 min | %Change |
|-------------------------|-----------------|-----------------------------|----------------|
| Placebo Trial 1A | 88.3 \pm 5.0 | 91.9 \pm 6.6 | 4 \pm 0.1 |
| IPC Trial 1A | 83.9 \pm 6.5 | 91.0 \pm 5.2 [*] | 8 \pm 0.1 |
| Placebo Trial 1B | 88.3 \pm 5.0 | 88.6 \pm 5.3 | 0.3 \pm 0.1 |
| IPC Trial 1B | 83.9 \pm 6.5 | 88.7 \pm 3.7 | 6 \pm 0.1 |
| Placebo Trial 2A | 85.8 \pm 6.0 | 89.4 \pm 5.9 | 4 \pm 0.1 |
| IPC Trial 2A | 83.9 \pm 7.8 | 87.7 \pm 4.5 | 4 \pm 0.1 |
| Placebo Trial 2B | 85.8 \pm 6.0 | 89.8 \pm 5.4 | 5 \pm 0.1 |
| IPC Trial 2B | 83.9 \pm 7.8 | 88.4 \pm 5.5 | 5 \pm 0.1 |

* Significant change from time point to immediately post-exercise.

Discussion

To our knowledge, this is the first study to examine the second window of protection of IPC and its effects on repeated cycling performance. We also believe it is the first investigation to examine how the second window of protection of IPC impacts recovery. The main findings of the study were: 1) there was a significant main effect of IPC treatment on TTF, and despite only finding significance between IPC trials 2A and 2B, mean performance time was greater and mean performance decrement was reduced with IPC despite varied individual performance, and that; 2) there was a significant main effect of IPC treatment on blood lactate levels, and subjects reached higher lactate levels in all trials with IPC treatment when compared to placebo. Lactate also appeared to recover at a similar rate with both treatments. Interesting secondary outcomes from the study were the positive effects of IPC treatment on heart rate and MAP. Post-trial heart rates with IPC treatment were on par with those in the placebo trials, despite higher workloads. The baseline MAP was lower at all IPC time points compared to placebo and, with the exception of one time point, MAP was also consistently lower after each of the IPC trials.

Performance- Time to Fatigue

The TTF results of the current study parallel results of a study performed by Kido et al., (2015), in which IPC improved

TTF by 4% in an incremental cycling test. They also found that lactate levels trended higher in the IPC group and that HR responses were on par with placebo, despite higher workload. They also found no difference in O₂ uptake, lactate, or RPE, but they did observe a 5.4± 4.8% greater maintenance of tissue saturation index in the IPC treatment group.

In a related crossover study²⁶, researchers had male cyclists perform a repeated 60-second supramaximal test, with a 45-minute period of passive recovery. They used a similar IPC protocol as the current study with 220 mmHg for the IPC treatment condition and 20 mmHg for the control condition. They found a 2% performance improvement, with no difference in VO₂ response. They also measured lactate throughout the passive recovery period and found that there was a significant change in the IPC treatment group when compared to the control trial. They concluded that an increased skeletal muscle activation and higher anaerobic contribution were enhanced by IPC in short-term exercise performance.

This is consistent with the results in a recently published study by Paradis-Deschênes et al. (2016), where they examined the performance and physiological responses of male endurance cyclists in repeated 5 km time trials. Their study was also a randomized crossover study with IPC (3 × 5 min ischemia/5-min reperfusion cycles at 220 mmHg) and

placebo (20 mmHg) administered to both thighs, but subjects were tested at simulated low (FIO₂ 0.180, ~1200 m) and moderate (FIO₂ 0.154, ~2400 m) altitudes. At the low altitude, the IPC group had improved 5 km time trial performance by 1.1% and at the moderate altitude, they improved their 5 km time trial performance by 1.5%. Similar to the current study, they also observed some variability, with some responders and non-responders. They also found that an increase in HR, SV and Q was driven by IPC treatment. They suggested the possibility that IPC acted directly on the cardiovascular response and that it may alter vasodilatory pathways and they concluded that IPC might be an effective strategy to enhance high-intensity endurance performance at a moderate altitude. Interestingly, the reported performance improvements in the aforementioned studies are comparable to the effects of a one-month 'live high — train low' altitude training program for elite runners²⁸.

However, these results conflict slightly with a study by Patterson et al. (2015), who performed a single-blind crossover study on male cyclists. The cyclists performed a series of 12 x 6-second sprints after receiving IPC (220 mmHg) or placebo (20 mmHg) treatment. They found that IPC treatment elicited a 2.4-3.7% increase in peak power for the first three sprints, but lead to no further change in the subsequent sprints. They also found no differences between trials for blood lactate, O₂ uptake, or RPE at any of the time points. They determined that

IPC might be beneficial for sprint activity, but not for endurance. This study is different in the cycling trial design, which might explain the difference in results when compared to the current study. As a single-blind study, perhaps there was also some bias on behalf of the subjects if they were aware of the treatment being received and therefore did not rate their perception of effort differently. A study by Crisafulli et al. (2011) also showed some conflicting results in their study on cyclists when compared to the present study. They had subjects perform an all-out supramaximal cycling bout following an IPC treatment, and at another time, without IPC treatment. Subjects were asked to perform at 130% of their established maximal W until exhaustion. Researchers found no difference in TTF between the treatments.

In summary, the studies that conflict with the current research are somewhat different in design since they were all examining the acute phase of IPC rather than the delayed phase. They were also sprint-based cycling studies that are more heavily reliant on the phosphagen system for ATP regeneration. Since there is a great degree of biovariability in how energy systems are impacted by genetics and training, this can help partially explain the degree of difference seen in the individual responses to IPC. Some of these studies also used passive recovery, which could certainly have an impact on the subsequent exercise bout. In a recent study investigating whether active or passive recovery was more beneficial to exercise

performance, researchers found that active recovery was more beneficial for maintaining endurance performance and sustaining power output³⁰. They also found that active recovery works best when performed at a moderate intensity (80- 90% or VT2, or 55-60% of heart rate reserve) to promote greater blood lactate removal and sustain endurance performance.

Based on the variability of individual responsiveness to IPC, the current study considered the importance of healthy endothelial function on IPC response, and the importance of active recovery to promote enhanced repeat performance. Looking at limitations from past studies, we controlled for risk factor burden through pre-screening and subject selection and controlled for the amount of training outside of the study, which knowingly can impact endothelial function. We also selected trained cyclists who were similarly fit, which might explain the potency of the results in this novel research into the delayed phase of IPC and its effects on performance and recovery. There was one male subject who performed better in the placebo trials and one female subject who performed similarly in the placebo and IPC trials. The male subject became a new parent just weeks before the IPC trials and was suffering from sleep deprivation and stress, which we believe impacted his performance. When he came back for the placebo trial four weeks later, he reported being better rested and that he'd been out biking in the preceding few weeks, which could also explain a better

performance. The female subject reported discomfort and bruising from the IPC treatment, which could have negatively impacted her performance.

Recovery- Lactate, HR, and MAP

There was a main effect of IPC treatment found at all time points and lactate levels were consistently higher in the IPC trials. Although subjects worked harder for a longer duration, their blood lactate levels appeared to recover similarly to the placebo, despite reaching higher levels at the end of the exercise bout. This is in keeping with the results found post-exercise in a study performed by Cruz et al. (2016) where they observed significantly higher lactate levels in the IPC treatment group. These results suggest that IPC treatments enhance the ability to clear, or to shuttle, lactate. A likely mechanism could be explained by an increased expression of monocarboxylate transporter proteins 1 (MCT-1) and 4 (MCT-4). Research by Juel et al. (2003) lead to the observation that these transporter proteins were expressed in erythrocytes following oxygen deprivation. MCT-1 proteins are found on Type I muscle fibres and favour the uptake of lactate as a fuel source for ATP production. MCT-4 proteins are found on Type II muscle fibres, which favour lactate efflux so that it can be better oxidized and used as fuel for glycolysis. These proteins play an important role in shuttling lactate to where it can be better utilized for fuel, and since lactate is shuttled on a 1:1 ratio with H⁺, this action also serves as a buffer, protecting the organism from metabolic

acidosis and subsequent fatigue. Since IPC exerts ischemic stress, it is plausible that MCTs are also expressed following IPC and have an impact on the lactate levels reached. With an increased expression of MCT-4, lactate could effectively be shuttled out to allow a high rate of glycolytic flux, while the MCT-1 allowed Type I fibres to take up lactate as a fuel and H⁺ are shuttled into mitochondria where they can be used to regenerate ATP in the electron transport chain. This proton consumption not only helps with ATP regeneration, but also effectively maintains pH balance and allows subjects to perform for a longer duration. Hypoxia also turns on hypoxia-inducible factor-1 alpha (HIF-1α), which is the master regulator of cellular and developmental response to hypoxia³². It controls the metabolic and pH-regulating pathways, causing cells to respond to hypoxia by upregulating glucose transporters GLUT-1 and GLUT-4. HIF-1 α also inhibits pyruvate from entering the TCA cycle, which leads to lactate conversion so that glycolysis can continue.

There was also a main effect of IPC treatment on heart rate and MAP at all time points. Indeed, HR responses were on par with the placebo trials, despite higher workload and higher blood lactate levels. The recovery rate was also similar across all trials. MAP increased significantly at only one point in the recovery period after Trial 1A, but was similar or lower than the placebo at all time points. The baseline MAP was also lower 24 and 48 hours after IPC treatment.

IPC has been known to lower HR and BP^{25,33}, which was certainly apparent in the current study. Although we did not measure stroke volume, we can infer that stroke volume increased as a result of IPC's influence on HR and vascular resistance. Since IPC is a known vasodilator due to stimulating the release of adenosine, bradykinin, and nitric oxide, it is likely that subjects experienced an increase in stroke volume, which permitted the heart rate values to remain comparable to placebo values despite higher workloads. Our subjects were able to perform for longer with IPC treatment and we can infer that with less vascular resistance, more oxygenated blood was able to circulate to skeletal muscle with each heartbeat.

Our hypothesis was that delayed IPC would be beneficial to both performance and recovery and our results show that IPC was indeed beneficial. IPC is a safe technique that can enhance performance and recovery, with no serious side effects. In this study, subjects experienced a 21% mean improvement in time to fatigue. These results with delayed IPC are better than other studies outlined above that noted a 3-5% improvement. This is on par, if not better than, the improvement demonstrated in other known ergogenic aids such as caffeine and erythropoietin (EPO)^{34,35}.

Caffeine is often considered a safe ergogenic aid by professional and amateur athletes alike. In a systematic review of caffeine as an ergogenic aid by Ganio et al. (2009), they found that the average improvement in

performance with caffeine ingestion was $3.2 \pm 4.3\%$ in endurance athletes. Since individual athletes vary in terms of caffeine metabolism, it is not a substance that all athletes can tolerate due to increased heart rate and digestive issues.

In comparison, there has been a lot of controversy over erythropoietin (EPO), which is a banned substance in competition. In one of the most notable studies on EPO and endurance performance, a small sample of non-elite cyclists were able to improve their time to fatigue by 54% due to a 12% increase in $\text{VO}_{2\text{max}}$ after a 14-day EPO protocol³⁵.

Since these were fit, but non-elite athletes, it was extrapolated that in an elite population, that performance increase might be closer to 5%. In contrast, a more recent study on elite athletes published in *The Lancet*³⁶ showed that although peak power and $\text{VO}_{2\text{max}}$ increased in the subjects who received EPO, their performance in a race setting did not differ significantly (0-3% time improvement). IPC shows good promise for safely enhancing performance and recovery, although there is much more to learn about effective dosage, individual response, and the protective window of delayed IPC.

Limitations

To our knowledge, this is the only study looking at the delayed phase of IPC and how it effects performance and recovery. We

controlled for menstrual cycle, activity level, caffeine intake, and also pre-screened our subjects to exclude those with any risk factor burden. Since the mechanisms behind individual variability and IPC responsiveness are still somewhat unclear³³, it would have been interesting to measure endothelial function and stroke volume. We had also initially planned to measure pH, but in the end, this was not possible. pH kinetics could have given us more knowledge about the recovery aspect of IPC, since that is still largely unknown.

Conclusions

In conclusion, IPC has shown performance enhancing benefits in different exercise modalities^{15,25,26,37,38} that coincide with the findings of the current study. Despite some individual variability, delayed IPC influenced TTF, blood lactate, HR, and MAP in repeated bouts of cycling at an altitude of 2350 metres in acclimatized, recreational cyclists. This novel research shows that delayed IPC has the ability to improve TTF, decrease performance decrement between treatments, maintain a comparable HR to placebo despite higher workloads, and lower MAP, which consequently, allowed cyclists to work at higher lactate levels. Further research is warranted to determine how long the protective effect of delayed IPC lasts. It would also be important to further investigate the dose-response relationship between IPC and performance to determine whether individuals respond better to an acute or delayed IPC treatment.

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