Optimizing the Ergogenic Use of Ischemic Preconditioning

by

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ABSTRACT

Optimizing the Ergogenic Use of Ischemic Preconditioning

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University of Guelph, 2019

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The overall purpose of this thesis was to develop the understating of how to best exploit the effects of Ischemic Preconditioning (IPC) on exercise performance, facilitating optimal application in athletics. To address this purpose, three specific research aims were implemented, and four independent studies were conducted. Study 1 & 2 aimed to investigate new variations of IPC administration to enhance the IPC stimulus and augment the ergogenic effect. It was found that enhancing the IPC stimulus, by amplifying the metabolic stimulus (study 1), or by combining the early and late IPC windows (study 2), does not augment the ergogenic effect of traditional IPC. However, an amplified metabolic stimulus may be beneficial for those who do not respond to traditional IPC. Study 3 aimed to examine the role of perception modulation as a mechanism responsible for the ergogenic effect of IPC. This was completed by investigating whether IPC could reduce sensitivity to a cold-water pain, and if the degree of pain reduction would relate to an individual’s improvement in exercise performance following exposure to IPC. These data revealed that IPC can reduce cold-water pain sensitivity; however, the magnitude and direction of changes were not related to IPC-mediated improvements in performance, suggesting reductions in pain sensitivity do not explain an individual’s ergogenic response following IPC. Study 4
aimed to evaluate the use of IPC as a tool that can be practically applied within traditional athletic training. It was determined that highly trained athletes undergoing consistent and repeated IPC treatment within their competitive training environment did not improve performance to a greater extent over time. This finding challenges the ecological validity of employing IPC as a supplemental ergogenic training strategy. Overall, these findings provide novel understanding into the optimal IPC methodology for evoking the ergogenic effect, mechanisms responsible for the IPC effect, and the feasibility of IPC within a competitive setting. These insights are important for optimizing IPC as an ergogenic strategy.
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To you Jenny, thank you for your love. It is the source of my motivation and the root of my happiness. I am excited for the next step in our lives together.
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<tr>
<td>AUC</td>
<td>Area under the Curve</td>
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<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
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<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<tr>
<td>EMS</td>
<td>Electrical Muscle Stimulation</td>
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<tr>
<td>HIF-1α</td>
<td>Hypoxia-inducible factor 1-alpha</td>
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<td>HR</td>
<td>Heart Rate</td>
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<td>IPC</td>
<td>Ischemic Preconditioning</td>
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<td>I-R</td>
<td>Ischemia-Reperfusion</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<td>NOS</td>
<td>Nitric Oxide Synthase</td>
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<td>LOP</td>
<td>Least Occlusive pressure</td>
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<td>PKG</td>
<td>Protein Kinase G</td>
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<td>Respiratory Exchange Ratio</td>
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<td>Reperfusion Injury Salvage Kinase Pathway</td>
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<td>SAFE Pathway</td>
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<td>$\text{VO}_2\text{max}$</td>
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<td>VE</td>
<td>Ventilation</td>
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Chapter 1 – Review of the Literature

1.1 Introduction to IPC

IPC is the application of transient, non-lethal episodes of ischemia and reperfusion to an organ or limb that activates protective mechanisms against subsequent ischemia-reperfusion (I-R) injury. IPC was first demonstrated in 1986 by Murry et al. (1), who found in a canine model that four, 5-minute alternate episodes of coronary artery occlusion and reperfusion were able to reduce the myocardial infarct size caused by a subsequent period of prolonged occlusion by 75%. Since this finding, IPC has been shown, in a laboratory setting, to exert cytoprotection against I-R injury in a wide variety of tissues, organs, and animal species (2).

Despite the cytoprotective properties of IPC treatment, clinical application was limited due to the invasive nature of applying ischemia and reperfusion directly to the tissue/organ system of interest. A major breakthrough came in 1993, as Przklenk et al. (3) demonstrated that applying IPC to one vascular bed in the heart reduced infarct size in an adjacent vascular bed that had not undergone IPC. This finding introduced the concept of “remote” IPC and allowed future investigations to demonstrate that cytoprotection could also be induced by applying IPC to distant or “remote” tissues (4), including a limb via a standard blood pressure cuff (5), rather than tissues directly. Ultimately, IPC represents a form of systemic protection against acute I-R injury.
Clinically, several studies have confirmed the benefits of IPC, as local and remote IPC has been used as a strategy to protect the heart against ischemic damage during cardiac surgery (6,7) and against ischemic injury during transplantation of the kidney (8,9). However, other studies demonstrate no clinical benefit of IPC (10–12).

The exact mechanisms through which IPC and remote IPC can induce cytoprotection against I-R injury remain poorly understood. To date, most IPC mechanistic research has been focused on the myocardium, as thousands of studies have reported >100 different signaling molecules involved in cardioprotection (13). However, the protective triggers, intracellular pathways, and effectors are thought to be very similar across tissues (14). The current paradigm suggests that the preconditioning I-R cycles cause an autocrine, paracrine, or distant (in the case of remote IPC) (15) release of trigger molecules (e.g. adenosine (16), bradykinin (17), opioids (18), nitric oxide (19)) that act on the cell at risk and activate a cytosolic mediator cascade(s) (i.e. NO/PKG pathway (20), the RISK pathway (21), SAFE pathway (22), HIF-1α (23)). During the sustained ischemia, this cytosolic signaling cascade transmits a protective signal onto the effector, which is the mitochondria (13). Ultimately, it is the activation of mitochondrial ATP sensitive potassium channels (24) and closing of mitochondrial permeability transition pores (25) during the sustained ischemia and early reperfusion that reduce mitophagy and attenuate cellular injury. Figure 1.1 provides a recently suggested signal transduction scheme for IPC-mediated cardioprotection.
IPC is known to protect skeletal muscle against I-R injury (26). In addition, IPC has been shown to elicit other metabolic adaptations in the skeletal muscle that include an improved oxidative capacity during prolonged ischemia (26–30), an improved post-ischemic blood flow (26,31,32), and improved muscle function following both ischemia and reperfusion (33,34). Based on these findings, many of these adaptations were hypothesized to yield improvements in exercise performance, as indeed, high-intensity exercise represents a form of ischemic-reperfusion insult (35) that could be amendable by IPC. As a technique that also involves the simple application of a blood pressure cuff to a limb, IPC has recently (<10 years) garnered a keen interest as a novel intervention to improve athletic performance.
Figure 1.1: Simplified scheme of cardioprotective signal transduction. The NO/PKG pathway is displayed in green, the RISK pathway in yellow, and the SAFE pathway in red. Akt indicates protein kinase B; AMPK, cyclic adenosine monophosphate–activated kinase; BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; Cx 43, connexin 43; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; FGF, fibroblast growth factor; Gs/Gi/o, stimulatory/inhibitory G protein; GPCR, G protein-coupled receptor; gp130, glycoprotein 130; GSK3β, glycogen synthase kinase 3β; H2S, hydrogen sulfide; H11K, H11 kinase; HIF1α, hypoxia-inducible factor 1α; IGF, insulin-like growth factor; iNOS, inducible NO synthase; IP3, inositoltrisphosphate; JAK, Janus kinase; KATP, ATP-dependent potassium channel; Na+/H+, sodium/proton-exchanger; NPR, natriuretic peptide receptor; pGC, particulate guanylate cyclase; p38, mitogen-activated protein kinase p38; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; PI3K, phosphatidylinositol (4,5)-bisphosphate 3-kinase; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; PTEN, phosphatase and tensin homolog; PTK, protein tyrosin kinase; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; SR, sarcoplasmic reticulum; STAT, signal transducer and activator of transcription; and TNFα, tumor necrosis factor α. Figure is reproduced with permission (License #4634770718751; see Appendix for details).
1.2 IPC and human exercise performance

Numerous studies have recently investigated the use of IPC application to a limb prior to an exercise bout in an attempt at improving exercise performance. The current literature on IPC and exercise has been extended to a drastic number of exercise modes, intensities, and durations, making assessment and discussion of the ergogenic effects difficult. Thus, for clarity, this review will divide the literature and discussion of the ergogenic effect of IPC into two exercise types: endurance exercise and sprint exercise. Endurance exercise is defined as an exercise task that requires a large aerobic contribution for energy production (maximal dynamic exercise > 90 seconds (36)), whereas sprint exercise is defined as an exercise task that does not require a large aerobic contribution, and instead relies on anaerobic glycolysis and the ATP-CP system for the majority of energy production (maximal dynamic exercise < 90 seconds and resistance exercise (36)).

1.3 The effect of IPC on endurance exercise performance

The effect of IPC on endurance exercise was first investigated in 2010 by de Groot et al. (37). This study found that applying three, 5-minute cycles of ischemia and reperfusion to both legs using blood pressure cuffs inflated at 220 mmHg increased maximal workload by 1.6% in an incremental cycling test to exhaustion. Likewise, Crisafulli et al. (38) observed increased maximal workload (4%) during a similar test after IPC. Findings of small but significant improvements in maximal workload following
IPC quickly progressed to reports of significant improvements in endurance time trial performance. IPC has been reported to improve 5-km running performance (2-3%) (39,40), 1-km rowing performance (1%) (41), and 5-km cycling performance (2%) (42). These time trial performance improvements (1-4%), albeit small, are practically meaningful. For instance, Jean-St-Michel et al. (43) reported a 1.1% improvement in 100 m swim time in national and international calibre swimmers. An improvement that, as the authors note, represented the expected adaptation following two years of training in highly competitive athletes. However, not all studies have corroborated these findings of improved endurance performance, as other studies demonstrate that IPC does not improve maximal workload during an incremental test to exhaustion (44), or time trial performance in 4-km cycling (45), 2-km rowing (46), or 1-km speed skating (47). As such, the current status of the ergogenic effect of IPC on endurance performance is still equivocal, and future work consolidating the types of endurance exercise and caliber of athletes which may benefit from IPC is required.

1.4 The effect of IPC on sprint exercise performance

The first study that investigated the effects of IPC on sprint exercise reported notable decreases in peak and mean watts during a 30 s anaerobic Wingate (cycling) test and suggested that IPC is detrimental to sprint exercise performance (48). However, Lalonde et al. (49) reported no change in peak and mean watts during the same test following IPC. Other studies have demonstrated beneficial effects of IPC on
sprint exercise, as mean and peak power output was enhanced during a 6 s cycling sprint (~3%) (50) and in the first 5 s of a 60 s cycling sprint (~7%) (51), however, beneficial effects of IPC on short duration cycling sprints (<10 s) are not consistently observed (49,52). Additionally, peak force production during maximal leg extensions is increased (10%) following IPC (53). Despite the potential beneficial effects of IPC on force and power output, improvements in sprint time trial performance have yet to be observed; previous studies have reported no improvements in 10-, 20-, or 30-m maximal running sprint time in team-sport athletes (54) or trained sprint athletes (45).

1.5 Mechanisms explaining the ergogenic effect of IPC

Although some studies found no effect of IPC on endurance exercise performance, much of the reported research suggests that IPC indeed induces small, but meaningful improvements (1-4%) to endurance exercise. Additionally, most studies found no effect of IPC on sprint exercise performance. To date, experimental evidence explaining the mechanisms responsible for the ergogenic effect of IPC is inconsistent and limited. The current evidence is briefly summarized into potential mechanisms in the following sections.

1.5.1 Skeletal muscle blood flow

Animal based investigations using prolonged ischemia have shown that IPC can improve post-ischemic skeletal muscle blood flow (26,31,32) by preserving microvascular function (56). In humans, IPC can protect against exercise-induced (57)
and ischemia-induced endothelial dysfunction (5,58), and enhance functional sympatholysis (59). These mechanisms likely mediate previously reported increases in skeletal muscle oxygenation during exercise following IPC (50,53,59). It has been demonstrated that nitric oxide (NO) plays a role in the IPC-mediated effects on endothelial function (60) and sympatholytic activity (61). Therefore, IPC-mediated release of NO (19) may improve skeletal muscle blood flow by preserving endothelial function, as well as attenuating sympathetic vasoconstriction on peripheral vasculature. A greater muscle blood flow and perfusion would act to improve exercise performance by augmenting oxygen delivery, oxygen extraction, and by limiting acidosis via removal of waste products (62). Indeed, VO₂max has been shown to increase following IPC (37). In contrast, some studies have demonstrated that IPC does not increase skeletal muscle blood flow during exercise (63,64) or improve VO₂max (38,39,44). More research is required to confirm or refute if IPC-mediated increases in skeletal muscle blood flow are involved in the ergogenic effect.

1.5.2  Skeletal muscle oxidative capacity

Animal work investigating the cytoprotective effects of IPC on skeletal muscle have demonstrated that IPC attenuates ATP depletion (26–28,30), glycogen depletion (29), lactate production (26,27), and mitochondrial dysfunction (65,66) during a period of prolonged ischemia. These results suggest IPC can improve the oxidative capacity of the skeletal muscle. However, evidence of IPC improving human skeletal muscle
oxidative capacity during exercise is limited. Bailey et al. (39) reported that IPC preceding an incremental running test allowed for a higher running velocity before the onset of blood lactate accumulation. IPC has been demonstrated to increase peak O$_2$ extraction during handgrip exercise to failure (64), moderate intensity cycling (67), and repeated maximal knee extensions (53). In addition, studies have also determined an acceleration of VO$_2$ kinetics at the onset of moderate intensity exercise following IPC (67,68), which would reduce the oxygen deficit (69). However, these adaptations may again be due to improved muscle blood flow and perfusion rather than an improved mitochondrial activation. Griffin et al. (70) demonstrated an improved cycling efficiency at the highest sustainable power output (critical power; >95% VO$_2$max) following IPC, which may again be the result of IPC-mediated release of NO (19), as nitrate supplementation has been shown to possibly improve mitochondrial and contractile efficiency (71,72). However, IPC does not seem to alter exercise efficiency at submaximal intensities (30-85% VO$_2$max) (45,73). Ultimately, the effect of IPC on skeletal muscle oxidative capacity requires further investigation.

1.5.3 Anaerobic capacity

In animal tissue, IPC maintained ATP content via increased anaerobic glycolysis (74), and PCr production (75) during prolonged ischemia. In humans, IPC increased anaerobic energy contribution during an improved 60 s cycling sprint performance, as accumulated oxygen deficit, amplitude of blood lactate, and total amount of O$_2$
consumed during recovery were augmented under unaltered VO₂ responses during exercise (51). However, several other studies have reported that IPC does not increase amplitude of blood lactate during improved endurance (38,70) or sprint exercise (50,53). Therefore, it remains unlikely that the ergogenic effect of IPC is mediated by an augmented anaerobic energy contribution.

1.5.4 Perception of effort

In skeletal muscle, thinly myelinated (group III) and unmyelinated (group IV) afferent neurons are sensitive to a variety of exercise-induced intramuscular metabolites, including lactate, ATP and protons (76–78). Feedback from these group III/IV muscle afferents can induce central fatigue (79) and inhibit α-motor neuron activation (80). It has been speculated that IPC may reduce the metabolic sensitivity of these skeletal muscle afferents, resulting in blunted fatigue signals to the central nervous system and improved performance during an exercise task (38,81). This hypothesis supports previous findings of an improved endurance performance accompanied by attenuated ratings of perceived exertion (39,82) and a progressive increase in the myoelectric activity throughout exercise (82).

Metabolically-sensitive muscle afferents also contribute significantly to hemodynamic responses through sympathoexcitation (80). If IPC reduces afferent feedback, impairments in the hemodynamic response and muscle sympathetic activity should be also be present during exercise. However, direct measures of muscle
sympathetic outflow and blood pressure were not reduced during static handgrip or post exercise circulatory occlusion after IPC (83). In addition, IPC was reported to increase systemic vascular resistance during post-exercise circulatory occlusion (84). These findings suggest IPC does not reduce feedback from group III/IV muscle afferents.

 Activation of opioid receptors by endogenous opioid peptides have been associated with the protective effect of either local or remote IPC in skeletal muscle (27,85). Therefore, it is possible that the IPC-mediated release of opioids could act on opioid receptors not only at peripheral sensory neurons, but also at the spinal cord and brain (86), resulting in a modulated perception of pain and improved performance during exercise (87).

### 1.6 IPC administration

#### 1.6.1 Time lag

The time lag between the completion of the IPC treatment and the start of the exercise has varied between studies. Studies have started exercise immediately (70), 5 minutes (37,88), 15 minutes (73), 30 minutes (41,64), 45 minutes (39,43), and 120 minutes (46) following the completion of the IPC treatment. The optimal time lag between IPC and the start of exercise is not known, and current literature offers no clear relationships with exercise performance improvements. IPC is known to exert cellular protection against I-R injury immediately following the completion of treatment and prolonged for 1-2 hours (58). Therefore, the optimal time lag between the completion of
IPC treatment and the start of exercise may be any time within this window. IPC is also known to exert a late window of I-R protection that appears at 12-24 hours following treatment, and lasts 48-72 hours (58). Previous studies have demonstrated that ergogenic effects can also be observed within this late window (40,89).

1.6.2 IPC protocol

The first IPC protocol employed before exercise involved three, 5-minute cycles of limb ischemia, followed by 5 minutes of reperfusion at a cuff pressure that caused complete blockade of arterial inflow (37). This protocol was based off of one previous study that showed 5 minutes of ischemia and reperfusion reduced the ischemic damage to rat anterior tibialis muscle to a greater extent than other I-R durations (90). To date, very few studies investigating the effects of IPC on exercise performance have deviated from this framework. Every study included in the systematic review by Incognito et al. (91) employed an IPC protocol of either three or four, 5-minute cycles of ischemia followed by 5 minutes of reperfusion at a pressure that occluded arterial inflow.

Research attempting new variations of the IPC protocol to enhance the stimulus and augment the ergogenic effect is limited (92,93). Increasing the number of cycles does not seem consequential to the ergogenic effect of IPC, as applying 8 cycles of 5-minute I-R offers no further benefit to performance (92). Additionally, a longer I-R cycle length of 10 minutes did not improve exercise performance (93). The volume of muscle mass subjected to the stimulus is also likely not related to the ergogenic effect. The
same magnitude of performance improvement is observed after bilateral IPC independent of whether the cuffs are placed on the legs or arms (92).

1.7 Potential sources of between-study variability

The results of this review indicate that IPC could act as a novel intervention to increase exercise performance, but the true efficacy still remains debatable. Nevertheless, it is clear that there is considerable variability between studies.

1.7.1 Exercise modality

Previous studies investigating the effect of IPC on exercise performance have used a wide variety of exercise modes. Comparing between studies, the ergogenic benefits of IPC have been investigated in cycling (38), rowing (41), running (39), speed skating (47), handgrip (64), knee flexion (53), and swimming exercise (43). As beneficial effects have been observed in most of these exercise modes, it may be that mode does not determine the ability of IPC to exert an ergogenic effect. However, no previous study has directly compared different modes of exercise using a within-subjects design.

1.7.2 Training status

Participant training status has greatly differed between studies. Using available individual data, Incognito et al. (91) reported that individual time-trial positive response rates were 81, 57, and 79% for highly trained, trained and recreationally active
population, respectively. However, no previous study has directly investigated the effect of training status on the ergogenic effect of IPC.

1.7.3 Pre-study restrictions

The current literature is inconsistent in limiting confounders known to modulate the effects of IPC, such as caffeine (94), alcohol (95) and physical activity (96). Furthermore, across the studies that do limit these confounders, restriction timelines are not standardized. Caffeine, alcohol, and physical activity restrictions have been implemented 2 hours to 48 hours prior to the IPC treatment (91).

1.7.4 Between-subject variability

A large between-subject variability has been observed in the exercise performance response to IPC. Incognito et al. (91) reported that upon examination of available individual responses in 9 studies that improved endurance time-trial performance, there were improvements in 107 participants and no effect in 43 participants, respectively (71% response rate). It has been suggested that the large variability in exercise performance response within studies may be explained by responders and non-responders to the IPC treatment (91,97). To date, explanations for differences in response to IPC remain unclear, however, previous studies have reported a reduced IPC-mediated cardio-protection in women, diabetics, and older patients with coronary artery disease (98,99). Additionally, a reduced IPC-mediated ergogenic effect
has been reported in women (52,89,100), suggesting a possible phenotypic explanation for responders and non-responders.

1.8 Conclusions

Ischemic preconditioning has a small but meaningful benefit on exercise performance. To date, the beneficial effect is mainly observed in endurance exercise, whereas sprint exercise seems less influenced by IPC. The exact mechanism explaining the ergogenic effect of IPC is unknown but may be related to an augmented skeletal muscle blood flow, improved oxidative metabolism, or alterations of perception during exercise. IPC administration traditionally involves three or four cycles of I-R at a cuff pressure that occludes arterial inflow, however the IPC protocol that optimizes the ergogenic effect has yet to be established. A large variability exists between studies, which may be owed to a large between-subject variability. Populations of responders and non-responders may explain the large between-subject variability, but reasons explaining a heterogeneous response are unknown.
Chapter 2 – Dissertation Aims and Specific Objectives

2.1 Overall Aims

Current literature suggests that IPC may be efficacious as an ergogenic aid to gain a competitive advantage for physical performance. As a method that is easily administered, non-invasive, and inexpensive, IPC represents an attractive tool that can easily be applied within an athletic environment. However, to date, the optimal methodology for maximizing the ergogenic effect and the mechanism responsible for the effect are unknown. Furthermore, research has been confined to a laboratory setting; thus, the ecological validity and feasibility of IPC use within an athlete’s normal training environment has not been established. Therefore, more research is needed in order to optimize the implementation of IPC within an athletic setting and facilitate the greatest possible competitive advantage. To this end, the aims of this thesis are to: I) Investigate new variations of the IPC administration in an effort to enhance the IPC stimulus and augment the ergogenic effect, II) Examine the role of perception modulation in the mechanism responsible for the ergogenic effect, III) Evaluate the use of IPC as a tool that can be practically applied within athletic training. To address all three aims of this thesis, four independent studies were conducted, which are outlined below.
2.2 Specific Study Objectives and Hypotheses

2.2.1 Aim I: Investigate new variations of the IPC administration in an effort to enhance the IPC stimulus and augment the ergogenic effect.

Study 1 (Chapter 3): It has been suggested that the release of endogenous metabolites is necessary to initiate the IPC stimulus. Amplifying this metabolic release or accumulation may enhance the IPC stimulus and augment the ergogenic effect. Therefore, the objective of this study was to enhance the IPC stimulus by combining IPC with either active walking or passive electrical muscle stimulation to amplify the metabolic effect evoked during the IPC treatment. It was hypothesized that IPC combined with muscle contractions induced by slow walking or electrical muscle stimulation would augment the ergogenic effect of traditional IPC.

Study 2 (Chapter 4): IPC is known to exert cellular protection against I-R injury for 1-2 hours following the completion of the IPC protocol. IPC is also known to exert a late window of protection on ischemic-reperfusion injury that appears at 12-24 hours following administration and lasts for 48-72 hours. Therefore, the objective of this study was to investigate whether the beneficial effects of early and late IPC windows can summate and further enhance the ergogenic stimulus and augment the effect of an isolated early IPC application. It was hypothesized that IPC administered in both the early and late windows would elicit an exaggerated performance response compared to IPC administered in the early window alone.
2.2.2 Aim II: Examine the role of perception modulation in the mechanism responsible for the ergogenic effect.

Study 3 (Chapter 5): IPC may enhance the production or release of endogenous opioids, which could act on opioid receptors not only at peripheral sensory neurons, but also at the spinal cord and brain, leading to an attenuated perception of pain and enhanced exercise performance. Therefore, the objective of this study was to investigate whether IPC could reduce pain sensitivity in healthy, young individuals, while also comparing IPC-induced changes in pain sensitivity to IPC-induced changes in 5-km cycling time trial performance. It was hypothesized that IPC would reduce non exercise-specific pain sensitivity, and that the degree of pain reduction would relate to the individual’s ergogenic response to IPC.

2.2.3 Aim III: Evaluate the use of IPC as a tool that can be practically applied within an athletic training environment.

Study 4 (Chapter 6): IPC represents an attractive potential ergogenic aid for athletes. However, current literature lacks a proper evaluation of IPC as a tool that can be practically applied within an athlete’s day-to-day training environment. Therefore, the objective of this study was to determine the impact of consistently, and repeatedly using IPC within an athlete’s training block for improving training benefit and maximal endurance performance over time. It was hypothesized that athletes who used IPC would have a greater improvement in endurance performance after 8 weeks of training compared with similar athletes who did not undergo IPC treatment.
2.3 Thesis declarations

This thesis includes four independent studies that have been previously published or prepared as peer-reviewed journal articles, and thus represents a “sandwich” thesis. Publication and co-authorship information can be found at the beginning of each applicable Chapter (Chapter 3-6). Permission to include copyright material has been obtained from the copyright holder. Permission details can also be found at the beginning of each applicable Chapter (Chapter 3, 4 & 6) and full details can be found in the Appendix. All experiments presented in this dissertation were conducted in accordance with the ethical guidelines of the University of Guelph and were approved by the University Research Ethics Board for Human Research.
Chapter 3:
Enhanced Metabolic Stimulus Stress Augments Ischemic Preconditioning for Exercise Performance

Presented as published:


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Co-authorship statement:

Joshua Slysz and Jamie Burr conceived and designed the research. Joshua Slysz conducted experiments, analyzed data, and wrote the manuscript.
3.1 Abstract

**Purpose:** To identify the combined effect of increasing tissue level oxygen consumption and metabolite accumulation on the ergogenic efficacy of ischemic preconditioning (IPC) during both maximal aerobic and maximal anaerobic exercise. **Methods:** Twelve healthy males (22 ± 2 years, 179 ± 2 cm, 80 ± 10 kg, 48 ± 4 ml kg\(^{-1}\) min\(^{-1}\)) underwent four experimental conditions: (i) no IPC control, (ii) traditional IPC, (iii) IPC with EMS, and (iv) IPC with treadmill walking. IPC involved bilateral leg occlusion at 220 mmHg for 5 minutes, repeated three times, separated by 5 minutes of reperfusion. Within 10 minutes following the IPC procedures, a 30 s Wingate test and subsequent (after 25 minutes of rest) incremental maximal aerobic test were performed on a cycle ergometer. **Results:** There was no statistical difference in anaerobic peak power between the no IPC control (1211 ± 290 W), traditional IPC (1209 ± 300 W), IPC + EMS (1206 ± 311 W), and IPC + Walk (1220 ± 288 W; P = 0.7); nor did VO\(_2\)max change between no IPC control (48 ± 2 ml kg\(^{-1}\) min\(^{-1}\)), traditional IPC (48 ± 6 ml kg\(^{-1}\) min\(^{-1}\)), IPC + EMS (49 ± 4 ml kg\(^{-1}\) min\(^{-1}\)) and IPC + Walk (48 ± 6 ml kg\(^{-1}\) min\(^{-1}\); P = 0.3). However, the maximal watts during the VO\(_2\)max increased when IPC was combined with both EMS (304 ± 38 W) and walking (308 ± 40 W) compared to traditional IPC (296 ± 39 W) and no IPC control (293 ± 48 W; P = 0.02). **Conclusion:** This study shows that in a group of participants for whom a traditional IPC stimulus was not effective, the magnification of the IPC stress through muscle contractions while under occlusion led to a subsequent
exercise performance response. These findings support that amplification of the ischemic preconditioning stimulus augments the effect for exercise capacity.

3.2 Introduction

It has been demonstrated that brief periods of circulatory occlusion and reperfusion, or ischemic preconditioning (IPC), can act to improve exercise performance (39,43). Multiple studies have demonstrated that IPC performed in the minutes to hours preceding aerobic (37) or anaerobic (50,51) exercise can improve performance but there appears to be great variability in response and, at present, the magnitude and consistency of the IPC effect across populations is not clear for neither aerobic (39,44,101) nor anaerobic (48,49) exercise. Contributing to the lack of clarity around IPC as an effective ergogenic aid is the fact that the physiological signaling stimuli and associated downstream responses remain incompletely characterized. Of the leading physiological theories, local hypoxia (leading to HIF1-α signaling (102)) and metabolite accumulation (such as adenosine, bradykinin, ROS, and opioids (103,104)) have received considerable attention; however, the existence of a dose-response relationship or identification of a threshold to trigger the biochemical pathways leading to the IPC effect remain unconfirmed (103,104). Given the many variations of IPC methodology reported in the current literature (i.e., differences in duration and number of cycles, occlusion pressure, volume of restricted muscle mass, local exercising, or remote muscle group), defining a pattern of the most efficacious method remains a challenge.
The metaboreflex is a key factor in controlling sympathetic outflow during exercise (105) and studies utilizing ischemia to amplify metabolites and provide increased afferent feedback have shown an elevated sympathetic outflow and blood pressure response (106,107). Provided that the accumulation of metabolites is adequate, IPC could promote metaboreflex induced increases in sympathetic outflow and blood pressure, preparing the body for subsequent exercise. IPC alone, however, has not been shown to elicit a sympathetic response, whereas the combination of cyclic bouts of blood flow restriction-reperfusion and treadmill exercise at 65% heart rate max has (108). It remains unclear if this combination can lead to improvements in performance, but it is possible that a sufficient metabolic stimulus (intramuscular perturbation) of IPC may be a crucial factor to elicit the desired effect.

By combining IPC with light exercise, such as walking, the muscle contractions thus evoked could function to amplify the hypoxic and/or metabolic preconditioning stimulus. As exercising while under blood flow occlusion may not be feasible or practical in certain situations (e.g., limited mobility during travel or when other temporal or spatial limitations exist in warm-up), the passive technique of electrical muscle stimulation (EMS) may be a more suitable option to similarly combine muscle contractions with IPC. Thus, we were interested in attempting to augment the IPC performance effect by combining IPC with either active walking or passive EMS to enhance the stimulus evoked during a single treatment session. Both the active and passive models represent
possible pre-competition strategies to increase tissue level hypoxia and metabolite accumulation compared with IPC alone.

Ischemic preconditioning is most commonly performed using supra-arterial occlusion pressures, dictating that both arterial inflow and venous outflow are subsequently restricted. As such there is a direct, and perhaps unavoidable, link between a greater emphasis on anaerobic metabolism and metabolite accumulation under these conditions which is challenging to meaningfully disentangle (109). Therefore, the purpose of this study was to identify the combined effect of increasing tissue level oxygen consumption and subsequent metabolite accumulation on the ergogenic efficacy of IPC during both maximal aerobic and maximal anaerobic exercise. It was hypothesized that IPC combined with muscle contractions induced by slow walking or electrical muscle stimulation would augment the ergogenic IPC effect, as demonstrated by greater aerobic and anaerobic power outputs.

3.3 Methods

3.3.1 Subjects

Twelve healthy males (22 ± 2 years, 179 ± 2 cm, 80 ± 10 kg, 47.7 ± 4 ml kg⁻¹·min⁻¹) volunteered to participate in this study which employed a randomized cross-over design. All participants were recreationally active non-smokers. Participants had no medical history of chronic disease and were safe to exercise as confirmed through completion of a PARQ+ screening questionnaire (110). This study was carried out in
accordance with the recommendations of the University of Guelph’s human ethics research board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Guelph’s human ethics research board (REB# 15SE019).

3.3.2 Protocol and measurements

All participants refrained from alcohol, caffeine, and intensive physical exercise for at least 24 h prior to testing. On each of four visits to the lab, participants performed both a 30 s anaerobic Wingate test with a standardized 5-minute warmup and warm-down and, after a 25-minute rest, a subsequent incremental maximal exercise test. Both tests were completed on a cycle ergometer (Velotron Inc., Seattle, WA, United States). The four experimental visits were performed at least 1 week apart and at the same time of day. Each visit involved either (i) baseline control involving no IPC, (ii) traditional IPC, (iii) IPC in combination with EMS, and (iv) IPC in combination with treadmill walking (2 mph at 0% grade). Ten minutes following the IPC procedures (described below), the performance tests were initiated as per the graphical representation of the protocol presented in Figure 3.1. To eliminate possible training, learning, or familiarization effects, all conditions were assigned in a random order. Participants, who otherwise had little in the way of expectations concerning the expected effects, were blinded to all performance data and were not informed a priori as to the expected outcomes of the study to avoid introducing possible placebo or nocebo effects.
3.3.3 Ischemic preconditioning

Ischemic preconditioning was performed prior to exercise in a seated position using bilateral arterial occlusion. The occlusion cuffs (Zimmer ATS 1500; United States) were positioned around the proximal thigh and inflated to 220 mmHg for 5 minutes. This procedure, which is most commonly used in the IPC exercise performance literature (91), promotes complete occlusion of both the arterial inflow and venous outflow in the lower limbs throughout the 5 minutes (111) as was confirmed in the present study using a near-infrared spectroscopy device (MOXY, MN, United States), and the
disappearance of a distal pulse. This ischemic procedure was repeated three times, each separated by 5 minutes of reperfusion (91). IPC in combination with EMS was also performed in a seated position and involved the above-mentioned IPC protocol with electrically evoked muscle contractions throughout. The EMS (Compex International, Mi-Runner Sport, United Kingdom) involved two surface electrodes placed on both the Vastus Medialis and Vastus Lateralis at the distal and proximal position that best elicited a muscular contraction. Stimulation was applied using a pulse train length of 400 µs, delivered at a frequency of 50–100 Hz at a maximally tolerable intensity level. As participants accommodated to the stimulation during a session, the stimulation intensity was progressively increased. IPC in combination with walking involved the above-mentioned IPC protocol with slow walking on a standard motor driven treadmill (Sole F63 treadmill, Canada) at 2 mph (112).

3.3.4 30 s Anaerobic Wingate test

The 30 s Anaerobic Wingate test included a “flying start,” which consisted of 40 s of low load (100 W) pedaling prior to the introduction of the resistance (7.5% body weight), against which participants aimed to maintain maximal pedal revolutions for 30 s. Integrated Wingate testing software was used to calculate peak power output in watts.
3.3.5 *Incremental maximal aerobic capacity test*

The incremental exercise test began with a resistance of 100 W and increased continuously at 1 watt every 3 s until exhaustion (i.e., the participant was unable to maintain a pedaling frequency of $\geq$50 rpm). Starting 1-minute prior, and continuing throughout the maximal exercise test, oxygen consumption ($\text{VO}_2$) was measured via indirect calorimetry using a face mask and optical turbine connected to a gas analyser with a sampling line (Cosmed Quark CPET, Rome, Italy). The maximal values were recorded as the highest reading that occurred after the data was smoothed using a rolling 30 s average. Attainment of true physiological max was confirmed for all subjects by the presentation of a plateau in $\text{VO}_2$ (increase in $\leq$50 mL/min at $\text{VO}_2$ peak and the closest neighboring data point), or respiratory exchange ratio (RER) $\geq$1.15 (113). During the graded exercise test, $\text{VO}_2$ at submaximal intensities were recorded and compared every 20 W between 120 and 200 W to investigate possible effects on submaximal exercise efficiency.

3.3.6 *Statistics*

A Shapiro–Wilk test was used to confirm normality of data, prior to analysis. Comparisons between conditions were performed using repeated measures ANOVA, with LSD post hoc tests, as was appropriate. Statistical analyses were conducted using SPSS software (version 25; IBM, Chicago, IL, United States), with differences
considered to be statistically significant at $P < 0.05$. All data is presented as mean ± SD, unless specified otherwise.

3.4 Results

3.4.1 30 s Anaerobic Wingate test

Peak anaerobic power was 1211 ± 290 W during the no IPC control and 1209 ± 300 W following traditional IPC. When IPC was combined with EMS and walking, peak anaerobic power was recorded to be 1206 ± 311 W and 1220 ± 288 W, respectively. There were no statistical differences between any groups ($P = 0.7$).

3.4.2 Incremental maximal aerobic capacity test

Baseline $\text{VO}_2\text{max}$ was 47.7 ± 4 ml.kg$^{-1}$·min$^{-1}$ and 48.4 ± 6 ml.kg$^{-1}$·min$^{-1}$ following traditional IPC. When IPC was combined with EMS and then walking, $\text{VO}_2\text{max}$ was recorded to be 49.1 ± 4 ml.kg$^{-1}$·min$^{-1}$ and 48 ± 6 ml.kg$^{-1}$·min$^{-1}$, respectively. There were no statistical differences between any groups ($P = 0.3$; Figures 3.2 A, B). Submaximal oxygen consumption increased as the test progressed from 120 to 200 W, but these increases in $\text{VO}_2$ every 20 W were similar in their pattern and magnitude across all conditions (Table 3.1). Peak power output, recorded at the point of exhaustion during the incremental maximal aerobic test, was 293 ± 48 W during the no IPC control and 296 ± 39 W following traditional IPC treatment. When IPC was combined with EMS and walking, peak power output increased to 304 ± 38 W and 308 ± 40 W, respectively (Figures 3.3 A, B). Statistical analyses revealed significant increases in peak power
output when combining IPC with EMS (P = 0.02) and walking (P = 0.03) compared to IPC alone. There were also significant increases in peak power output when combining IPC with EMS (0.04) and walking (P = 0.002) compared to the control group.

Figure 3.2: (A) Mean maximal oxygen uptake ml·kg⁻¹·min⁻¹ from the incremental maximal aerobic test for each intervention and baseline. (B) Maximal oxygen uptake ml·kg⁻¹·min⁻¹ from the incremental maximal aerobic test for each intervention and baseline. Data is presented as mean ± SE.
Figure 3.3: (A) Mean peak watts from the incremental maximal aerobic test for each intervention and baseline. Data is presented as mean ± SE and the differences were considered significant at P ≤ 0.05. *Represents statistically different from baseline; #Represents statistically different from IPC alone. (B) Individual peak watts from the incremental maximal aerobic test for each intervention and baseline.

Table 3.1: Oxygen consumption at submaximal exercise intensities during an incremental cycling test after no intervention (Control) ischemic preconditioning (IPC), ischemic preconditioning combined with electrical muscle stimulation (IPC + EMS), and ischemic preconditioning performed during slow walking at 2 mph (IPC + Walk).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IPC</th>
<th>IPC + EMS</th>
<th>IPC + Walk</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ 120W (ml O₂·kg⁻¹·min⁻¹)</td>
<td>25 ± 3</td>
<td>25 ± 4</td>
<td>25 ± 3</td>
<td>25 ± 2</td>
<td>0.9</td>
</tr>
<tr>
<td>VO₂ 140W (ml O₂·kg⁻¹·min⁻¹)</td>
<td>28 ± 2</td>
<td>27 ± 4</td>
<td>28 ± 3</td>
<td>27 ± 3</td>
<td>0.5</td>
</tr>
<tr>
<td>VO₂ 160W (ml O₂·kg⁻¹·min⁻¹)</td>
<td>30 ± 4</td>
<td>30 ± 4</td>
<td>30 ± 2</td>
<td>30 ± 3</td>
<td>0.8</td>
</tr>
<tr>
<td>VO₂ 180W (ml O₂·kg⁻¹·min⁻¹)</td>
<td>33 ± 4</td>
<td>32 ± 4</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>0.5</td>
</tr>
<tr>
<td>VO₂ 200W (ml O₂·kg⁻¹·min⁻¹)</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
<td>36 ± 3</td>
<td>35 ± 3</td>
<td>0.8</td>
</tr>
</tbody>
</table>
3.5 Discussion

The present study sought to compare the effects of traditional IPC with an enhanced preconditioning stimulus, involving IPC combined with EMS or walking, for augmenting either aerobic and anaerobic performance. The main novel findings were that (I) IPC, when combined with walking or EMS significantly improved peak watt output in the maximal aerobic test to exhaustion, despite traditional IPC causing no significant benefit; (II) neither IPC nor an augmented adaptation of IPC improved maximal oxygen consumption; (III) neither IPC alone nor augmented IPC improved maximal anaerobic power. These findings suggest that a certain magnitude of metabolic and/or hypoxic stimulus may, thus, be important for stimulating the positive effects of IPC on exercise capacity, but that this effect was not driven by a change in aerobic or anaerobic maximal capacity.

3.5.1 Exercise performance

Previous studies have demonstrated increases in cycling peak power output (of 1.6–3.7%) following IPC treatment during maximal tests (37,38). The current data demonstrate traditional IPC to be ineffective for increasing peak power output during a maximal cycling test; however, when IPC was augmented with either passive twitches or active light-intensity muscular contractions, power output thereafter increased. More specifically, we observed a 3.8% increase in power with the addition of EMS to IPC and a 5% increase in power when slow walking was performed during the IPC treatment.
The effect of an 11–15 W increase in max power could be quite meaningful in a competition situation, and when modeled using the current participants’ weight and the assumption of zero grade and wind while cycling, these augmentations in power would be expected to result in a 0.5–0.7 kph improvement in speed (114). While it is difficult to compare directly the muscular stress while under IPC, it is likely that the added stress of walking was greater than that of EMS. It is also likely that this utilized additional muscle mass, thus, the increased efficacy with walking is logical. Furthermore, it was observed that of the 12 participants, 8 did not initially demonstrate improvements in power output with traditional IPC. However, when a greater metabolic stress was imposed, 7 of the 8 “non-responders” became “responders” and increased maximal power output, which is in line with previous evidence that a greater physiologic stimulus reduces the rate of non-response to a given perturbation (115). Comparing to previous literature, it is worth noting that the one study which previously reported no change in peak power output during cycling following IPC also used the lowest occlusion pressure (44), and it is thus possible that the induced metabolic stress was lower, similar to the pattern we report here.

In line with the current model of increasing the accumulation of metabolic waste products, Crisafulli et al. (38) have similarly attempted to magnify this effect by occluding leg circulation (for 3 minutes) immediately following submaximal cycling exercise. In partial agreement with our findings, this group reported that IPC
consistently increased peak power output compared to a control test; however, augmenting the metabolic stress through a post-exercise occlusion did not demonstrate further benefit compared to traditional IPC. This may suggest that the initial IPC provided a sufficient stimulus to elicit an optimal performance effect, or that the addition of a brief 3-minute augmented IPC period was insufficient to further amplify the response. In our study, in which we invoked muscle contractions throughout all cycles of the IPC, this stress was prolonged and repeated and may account for the differences in response. While we did not observe efficacy of traditional (using similarly matched) IPC protocol and graded cycling test with male participants, the addition of metabolic stress led to a response of similar magnitude. The discrepancy regarding the efficacy of traditional IPC between studies may be attributable to IPC protocol differences or participant training status, as subjects in the current study reached \(\sim 10\%\) higher peak watts, and thus a higher threshold of metabolic stress during IPC may have been required to elicit a similar response. The specific role of training status on the efficacy of IPC for affecting exercise performance requires further study.

3.5.2 Maximal aerobic capacity

As is consistent with the majority of other studies, compared to the control group, there was no increase in VO\(_{2}\)max after traditional IPC (39,44,101). Despite improvements in exercise performance (peak watts), when metabolic IPC stress was augmented with the addition of either passive or active muscle contractions VO\(_{2}\)max
remained unaltered compared to the control. This suggests that performance gains are not the result of an increase in maximal capacity. There was also no change in submaximal VO₂ during cycling following traditional IPC, or following IPC combined with EMS or walking. This too is consistent with current literature (88) showing no change with traditional IPC, while also providing evidence that increasing the magnitude of the metabolic stimulus during IPC may have no effect on submaximal efficiency. Interestingly, 4 weeks of applying IPC after sprint interval training has been shown to increase VO₂max (116), suggesting an augmented IPC stress may be important in long-term aerobic adaptation rather than a short-term change. It must be recognized that it is possible our VO₂ measures were affected by a preceding anaerobic test. If true, this is a potential explanation for the disagreement between a previous study (37) that reported an increase in VO₂max with traditional IPC; however, this is unlikely as our findings are consistent with the majority of the existing literature (39,44,101)

3.5.3 Anaerobic capacity

Using a standard 30 s anaerobic Wingate test, there was no change in anaerobic peak power following traditional IPC or following IPC combined with EMS or walking. This finding agrees with previous studies (48,49) showing no ergogenic effect of IPC on anaerobic exercise, while also providing novel evidence that the magnitude of the metabolic stimulus during IPC may have little impact on anaerobic exercise. A select few studies have shown a beneficial effect of IPC on anaerobic exercise (50,51), with
positive effects typically occurring when IPC is employed further in advance (i.e., 30–60 minutes) of the exercise test; whereas studies that showed reduced or unchanged anaerobic performance used shorter periods (i.e., 5–15 minutes) (48,49) between IPC and the exercise test. Of note, the anaerobic test used in the current study occurred in the shorter time frame. The specific role of timing on the efficacy of IPC for affecting maximal anaerobic capacity needs to be further investigated. In addition, the studies that have shown positive effects of IPC on anaerobic exercise (50,51) appear to employ longer anaerobic effects (≥60 s) compared to the studies (48,49) that show no effect (30 s). The current study did not show any changes in peak or average power with IPC during the first or last 10 s of the 30 s Wingate test, suggesting that IPC does not assist with short-term energy provision. It is possible that IPC assists with energy provision with longer anaerobic efforts, but this remains speculative and requires further investigation.

The specific mechanism by which IPC works remains unclear. It is possible that the combination of IPC and rhythmic muscle contractions sufficiently altered local oxygen and metabolites to activate afferent feedback leading to a metaboreflex-induced sympathetic response during exercise, while IPC alone did not. This increase in sympathetic activity to non-active muscle could lead to greater blood flow and perfusion of the active muscle beds (117), and if preconditioning were performed locally, the proper distribution of blood flow could be further aided by sympatholysis during
Nevertheless, we observed no change in whole body VO$_2$max. An alternative explanation may be that IPC permits an enhanced central motor efferent command by attenuating inhibitory signals originating from metabolic sensory muscle afferents (38). This would, thus, allow participants to exercise slightly beyond their individual critical threshold of exhaustion for the exercise, which fits with our finding of increased power. Indeed, a complete blockade of muscle afferent feedback during exercise, using an intrathecal administration of fentanyl, results in large increases in central motor drive and power output (118). Cruz et al. (82) have observed an increase in aerobic energy provision with IPC, possibly reducing the utilization rate of anaerobic energy stores, lowering fatigue signals and delaying exhaustion. While the current study also does not offer any mechanistic insight, future studies will need to include more invasive measurements of blood flow, oxygen delivery, and arteriovenous oxygen difference across the working limb to determine whether IPC results in tissue specific improvements in these variables, which may be responsible for small improvements in peak watts.

### 3.6 Limitations

As with most performance research, there were potential limitations to the current study that should be recognized. The inclusion of a sham control for each IPC intervention was omitted, both for practicality and to avoid introducing a potential training effect of excessive repeated testing of the same subjects. As such, it is possible
that that a placebo effect could have occurred, if participants believed the treatment would help. However, participants were naïve to the expected treatment outcomes and it is conceivable that placebo effects were no more likely to occur than nocebo effects. It is undeniable that this area of research, as a whole has struggled to find an effective sham control, and while previous research has used low-pressure sham conditions in which the cuff is only inflated to 10–20 mmHg (39,43), this low pressure is easily distinguishable from true IPC. In addition, it is still unknown if the low-pressure itself can elicit a preconditioning response, thus we chose not to employ this technique in the current study and compared to a simple control condition. It is important to again recognize that the measures of the aerobic test (i.e. VO₂, peak watts) may have been affected by the preceding anaerobic test as the 30s Wingate test may have reduced NO availability and diminished the effects of IPC on the incremental test to exhaustion. Finally, the current study was conducted with participants that are young and recreationally active, thus, the relevance of these interventions in an athletic or clinical population remain to be tested.

3.7 Conclusion

In a group of participants for whom a traditional IPC stimulus was not effective, the amplification of an IPC stress through muscle contractions while under occlusion led to a subsequent increase in exercise performance. These findings support the hypothesis that there needs to be a sufficient metabolic and/or hypoxic stimulus for IPC to elicit an
ergogenic action. From a practical standpoint, the addition of either passive or active muscle contractions to the standard IPC protocol of 3 sets of 5-minute cycles of occlusion and reperfusion, may improve the efficacy and decrease “non-response” to IPC treatment, and this highlights that new variations of the IPC protocol should be explored in an effort to optimize the desired effect. Thus, augmenting the metabolic or hypoxic stress through muscle contractions may be an important and functional way to ensure the required metabolic/hypoxic stimulus is met for IPC to improve exercise capacity.
Chapter 4:

An Examination of Individual Responses to Ischemic Preconditioning and The Effect of Repeated Ischemic Preconditioning on Cycling Performance

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Co-authorship statement:

Joshua Slysz, Jade Marrow, and Jamie Burr conceived and designed research. Joshua Slysz and Heather Petrick conducted experiments and analyzed data. Joshua Slysz wrote the manuscript.
4.1 Abstract

**Purpose:** To use repeated control trials to measure within-subject variability and assess the existence of responders to ischemic preconditioning (IPC). Secondly, to determine whether repeated IPC can evoke a dosed ergogenic response. **Methods:** Twelve aerobically fit individuals each completed three control and three IPC 5-km cycling time trials. IPC trials included: *i)* IPC 15-min preceding the trial (traditional IPC), *ii)* IPC 24-h and 15-min preceding (IPCx2), *iii)* IPC 48-h, 24-h, and 15-min preceding (IPCx3). IPC consisted of 3x5-minute cycles of occlusion and reperfusion at the upper thighs. To assess the existence of a true response to IPC, individual performance following traditional IPC was compared to each individual's own 5-km TT coefficient of variation. In individuals who responded to IPC, all three IPC conditions were compared to the mean of the three control trials (CONavg) to determine whether repeated IPC can evoke a dosed ergogenic response. **Results:** 9 of 12 (75%) participants improved 5-km time (-1.8±1.7%) following traditional IPC, however, only 7 of 12 (58%) improved greater than their own variability between repeated controls (true responders). In true responders only, we observed a significant mean improvement in 5-km TT completion following traditional IPC (478±50 s), IPCx2 (481±51 s), and IPCx3 (480±49 s) compared to mean CONavg (488±51s; p<0.006), with no differences between various IPC trials (p>0.05). **Conclusion:** A majority of participants responded to IPC, providing support for a meaningful IPC-mediated performance benefit. However, repeated bouts of IPC on consecutive days does not enhance the ergogenic effect of a single bout of IPC.
4.2 Introduction

Ischemic preconditioning (IPC) is the exposure of brief periods of circulatory occlusion and reperfusion to a limb that activates protective mechanisms against ischemic-reperfusion injury in local and distant tissues (5,27). IPC applied prior to exercise has been shown to improve exercise performance, both when three or four cycles of 5-minute bouts of circulatory occlusion and reperfusion are applied to exercising (37,38) and non-exercising limbs (43,64). To date, much of the reported research suggests that IPC induces small, but meaningful improvements (1-4%) to endurance exercise (91); however, there appears to be a great variability in this response. A large between-subject variability has been reported in the exercise performance response to IPC, which confounds comparison of group-level means, and likely contributes to the large inter-study variation in response. It has been suggested that the large variability in exercise performance response within and between studies may be explained by responders and non-responders to IPC treatment (91,97). However, few existing studies that suggest individual responses to IPC employ appropriate experimental design (119), thereby preventing legitimate evaluation of response versus non-response to the IPC stimulus (120,121). It is imperative to assess the within subject variability in the exercise performance for an evaluation of true IPC response versus non-response. As the performance of an individual who undergoes an exercise trial is subject to motivational, biological, or measurement variation from bout
to bout, an improvement cannot be reliably characterized as a response unless the effect is greater than the typical variation associated with the exercise task.

It has been suggested that the effect of IPC could be influenced by the timing and distribution of when the IPC treatment is applied (122). This theory stems from the notion that IPC is capable of eliciting two phases of protection against cellular ischemic reperfusion injury; specifically, an early window of protection that appears immediately following the onset of IPC and extends for 1-2 hours (58) and a late window that appears at 12-24 hours following treatment and lasts 48-72 hours (123). Physiologically, it is believed that brief ischemia and ensuing reperfusion result in the transient generation of metabolites capable of triggering the early window of protection (124) and activating the potent cellular signaling molecule nitric oxide synthase (NOS) (125). In response to this acute stimulus, NOS triggers a complex signaling cascade to activate transcription factors and upregulate gene expression, thereby augmenting cellular adaptations during a delayed second window of protection following IPC treatment (126). As such, guiding the timing of IPC application to synergistically combine the effects of the early and late window, may therefore maximize the ergogenic effect of IPC. Previous studies have demonstrated that IPC can exert ergogenic effects within both the early and late window (40,89), but no study has examined the additive effect of combining the early and late windows. In addition, a recent study (122) administered three sessions of IPC to elite swimmers over two consecutive days prior to repeated sprint swimming performances.
(IPC 48-h, 24-h, and 30-min prior), a temporal sequence which aligns with both the early and late IPC windows. While a significant improvement in swimming performance was observed following IPC sessions, this study design did not provide a direct comparison between repeated IPC bouts and a traditional, single IPC administration. It, thus, remains unknown whether the beneficial effects of early and late IPC windows can summate and further amplify the ergogenic stimulus of an isolated IPC application.

To date, no study has established the existence of IPC-mediated performance responses that exceed day-to-day variation around the exercise task. Therefore, in the present study, we first aimed to use repeated control trials to measure within-subject variability, and as a result, assess the existence of responders to IPC. Secondly, we aimed to determine if IPC administered in both the early (15-min prior) and late (24-hr and 48-hr prior) windows is capable of eliciting a dosed and exaggerated ergogenic response. As differences in individual response to IPC may mask a dosed effect by introducing large variability, it was of particular interest to investigate a potential dosed response from repeated IPC in individuals specifically identified as responders. This approach will allow for a stronger conclusion to be made surrounding the efficacy of this technique. Therefore, it was hypothesized that I) IPC mediated performance responses would exceed day-to-day variation around the exercise task in certain individuals, II) IPC administered in both the early and late windows would elicit an exaggerated
response compared to IPC administration in the early window alone, but only in individuals identified as responders.

4.3 Methods

4.3.1 Subjects

Twelve aerobically trained individuals (30 ±7 years, 7/5: male/female, 175 ±13 cm, 74 ±13 kg, 55 ±6 ml·kg⁻¹·min⁻¹) volunteered to participate in this study which employed a randomized cross-over design. Participants were non-smokers, had no medical history of chronic disease and were safe to exercise as confirmed through completion of a PARQ⁺ screening questionnaire (110). After being advised of the purpose and potential risks of the study, subjects provided written informed consent in accordance with the guidelines of the institutional human ethics research board who approved the experimental protocol and procedures of this study (REB No. 17-08-034).

4.3.2 Protocol and measurements

Participants refrained from alcohol, caffeine, and intensive physical exercise for a least 24-h prior to performance testing. Each participant underwent seven separate 5-km cycling time trials. Specifically, one familiarization trial, three identical control trials (no IPC), and three different experimental IPC trials were conducted. Repeated control trials were completed for a calculation of each participant’s variability in performance when conducting repeated 5-km cycling time trials, in the absence of any intervention. Using a random number generator, all control and experimental IPC trials were
completed in a randomized order, with approximately 5 days in between each trial to allow sufficient recovery time.

During the initial familiarization visit, participants completed a maximal oxygen consumption test (VO₂max test) on a cycle ergometer (Velotron Inc, Seattle USA) to evaluate fitness. Expired gases were measured via indirect calorimetry using a face mask and optical turbine connected to a gas analyser with a sampling line (Cosmed Quark CPET, Rome, Italy). The maximal values were recorded as the highest reading that occurred after the data was smoothed using a rolling 30 s average. Attainment of true physiological max was confirmed for all subjects by the presentation of a plateau in VO₂ (increase in ≤50 mL/minute at VO₂ peak and the subsequent data points), or respiratory exchange ratio (RER) ≥ 1.15 (113). Immediately following the VO₂ max test, participants completed a familiarization 5-km TT to acquaint themselves with the gearing functions of the cycle ergometer and pacing strategies for the 5-km distance. Finish time was not recorded.

The three control trials were identical and were not preceded by IPC, only a standardized warmup (below). The three experimental IPC trials included: i) IPC completed 15-min preceding the trial (traditional IPC), ii) IPC completed 24-h and 15-min preceding the trial (IPCx2), iii) IPC completed at 48-h, 24-h, and 15-min preceding the cycling trial (IPCx3).
4.3.3 5-km cycling time trial performance

All cycling time trials were completed on a stationary cycle ergometer using an electromagnetic resistance (Velotron Inc, Seattle USA). Integrated 3D Computer Software was used to complete a flat, 5-km race, wherein time to completion (s), average and peak power output (Watts), and average revolutions per minute (RPM) were recorded. The system allowed participants to virtually change gears and select their own resistance/RPM to suit their desired pacing strategy. On a visible computer monitor participants viewed the image of a single rider on course (representing the test subject), but were blinded for elapsed time, speed, power output, RPM and gearing level, as these variables were removed from the software’s display interface during each trial. Participants were also blinded to the precise distance covered, however a general guide using a linear scale and representative marker was available for their reference to allow for pacing their work output towards the known endpoint of 5-km. The 5-km distance was chosen based on previous research that suggests the most consistent benefit of IPC is for an improvement in time-trial performance in exercise tests of aerobic capacity (91). In addition, this distance does not involve considerable pacing strategy; thus, this distance was also chosen to minimize any potential pacing variability or motivational influence commonly occurring during a prolonged cycling task. Participants were naïve to the expected treatment outcomes and received no verbal encouragement throughout any cycling test in order to avoid being influenced by the researchers. Prior to all trials, participants completed a 5-minute warm-up at a power
output of 100W on a cycle ergometer. For experimental IPC trials, IPC was started 40-min and completed 10 minutes prior to the standardized warm-up.

4.3.4 Ischemic preconditioning

Ischemic preconditioning was performed in a sitting position using bilateral arterial occlusion of the legs. Occlusion was accomplished using a PTSi automated tourniquet system (Defli Medical Innovations Inc. Vancouver, Canada). The tourniquet cuffs (11.5 cm width) were positioned proximally on the thighs and inflated to a pressure that was minimally superior to femoral systolic pressure ($\geq$ 2 mmHg), allowing complete arterial occlusion. Pressures ranged from 180-290mmHg. This pressure, called the lowest effective occlusion pressure (LOP), can be detected by the Delfi system for each participant by utilizing a pressure transducer to determine the pressure required to cause the arterial pulsation to disappear (127). LOP was determined in duplicate and the average of these two values was used to set the pressure for circulatory occlusion. Circulatory occlusion lasted 5 minutes and was performed 3 times, each separated by 5 minutes of reperfusion (91,128). No participant reported undue pain or discomfort during the leg circulatory occlusion.

4.3.5 Data analysis and statistics

To assess the existence of true performance responders to IPC, individual performance following traditional IPC on a 5-km cycling TT were compared to the same individual’s 5-km cycling TT typical variability. Within-individual variability was
represented as the coefficient of variation (CV) calculated from each participant’s 3 control trials. CV expresses the standard deviation (SD) of the measure as a percentage of the mean and is the appropriate measure for quantifying variability of athletic performance (129). Individual response to traditional IPC was represented by percent change relative to the mean of his or her 3 control trials, and if a participant’s response was improved to a magnitude greater than his or her own CV, that participant was classified as a “verified responder”. If a participant’s response was not outside one’s own CV, that participant was classified as a “non-responder”.

To determine whether repeated IPC can evoke a dosed, or exaggerated, ergogenic response, all three IPC experimental conditions; traditional IPC, IPCx2, and IPCx3, were compared to the mean of the three control trials (CONavg). Comparisons between conditions were performed using repeated measures ANOVA, with LSD post-hoc tests, as was appropriate. Statistical analyses were conducted using SPSS software (version 25; IBM, Chicago, IL, USA), with differences considered to be statistically significant at P < 0.05. Data is presented as mean ± SD, unless specified otherwise.

4.4 Results

4.4.1 Existence of IPC responders

There was no difference in participant’s 3 control trials when controlling for trial order (p=0.3), suggesting CONavg and CV were not influenced by a learning effect.
Comparing CON_{avg} to traditional IPC, the mean performance improvement over the 5-km TT did not reach significance (1.0 ±1.8 %, p=0.08, Cohen’s d=1.1, Table 4.1), despite a 0.6 % change being recognized as a meaningful alteration in highly-trained cycling performance (130). In addition, there were no differences in peak or mean power output, cadence, or velocity between all time trials (Table 4.1). An examination of individual participant data revealed 9 of 12 participants (75 %) decreased 5-km TT time (-1.8 ±1.7 %) with the remaining 3 of 12 participants (25 %) increasing 5-km TT time (+0.6 ±1.3 %) following traditional IPC (Figure 4.1). However, if a performance improvement was only considered true when a participant’s individual IPC response exceeded one’s own percent CV from repeated controls (group average CV 0.4 ±0.8 %; individual participant’s CV represented as hatched bars, Figure 4.2) then 7 (58 %) would be classed as verified responders and 5 (42 %) as non-responders (Figure 4.2).
Figure 4.1: Individual changes in the time to complete a 5-km cycling time trial in well-trained cyclists. Data presented as the mean of three control trials (CONavg) and traditional IPC (15-min preceding exercise). Responders and non-responders are represented by the smooth and dotted lines, respectively.

Figure 4.2: Histogram representing the magnitude of effect of traditional IPC on 5-km time trial performance, represented as percent change from each participant’s own control (white bars). The coefficient of variation of each individual’s three control trials are superimposed, represented in the hatched bars. Dotted lines represent the smallest worthwhile change of 0.6%.
4.4.2 Summed effect of the early and late window

When examining the temporal association of IPC application, there were no differences in mean 5-km TT completion time between CONavg, traditional IPC, IPCx2, or IPCx3 ($p=0.19$; Cohen’s $d=0.8$; Figure 4.3A; table 4.1). However, when examining only those whose traditional IPC response exceeded their own CV (verified responders, n=7) to establish if a dosed ergogenic effect exists after repeated IPC, there was an improvement in 5-km TT completion time following traditional IPC (478 ±50 s), IPCx2 (481 ±51 s), and IPCx3 (480.5 ±49 s) compared to CONavg (488 ±51 s; $p=0.006$; Cohen’s $d=2.1$), but no experimental IPC conditions were significantly different from each other ($p > 0.05$; Figure 4.3B). Furthermore, in verified responders (n=7) to traditional IPC, individual responses to IPCx2 and IPCx3 exceeded one’s own percent CV 81% of the time. When examining only those whose traditional IPC response did not exceeded their own CV (verified non-responders, n=5), there was no difference in 5-km completion time between CONavg (484 ±57 s), traditional IPC (487 ±60 s), IPCx2 (483 ±48 s), IPCx3 (482 ±46 s) ($p=0.8$; Cohen’s $d= 0.5$; Figure 3C), and 87 % of all IPCx2 and IPCx3 trials confirmed no improvement from control CV.
Table 4.1: Values of power (watts), cadence (rpm), velocity (km/h) and total time to completion (seconds) during a 5-km cycling time trial for three identical control trials and three experimental IPC trials. The three experiment IPC trials included: i) IPC completed 15-min preceding the trial (traditional IPC), ii) IPC completed 24-h and 15-min preceding the trial (IPCx2), IPC completed at 48-h, 24-h, and 15-min preceding the cycling trial (IPCx3). Data are presented Mean ± SD. * represents P < 0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Power (watts)</th>
<th>Cadence (rpm)</th>
<th>Velocity (km/h)</th>
<th>TIME (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Mean</td>
<td>Peak</td>
<td>Mean</td>
</tr>
<tr>
<td>Control Average</td>
<td>371 ± 95</td>
<td>278 ± 66</td>
<td>110 ± 10</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>Control Trial 1</td>
<td>364 ± 85</td>
<td>273 ± 66</td>
<td>111 ± 10</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>Control Trial 2</td>
<td>368 ± 96</td>
<td>279 ± 67</td>
<td>108 ± 10</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>Control Trial 3</td>
<td>381 ± 108</td>
<td>281 ± 70</td>
<td>110 ± 11</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>Traditional IPC</td>
<td>418 ± 108</td>
<td>287 ± 76</td>
<td>107 ± 8</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>IPCx2</td>
<td>407 ± 102</td>
<td>285 ± 72</td>
<td>111 ± 8</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>IPCx3</td>
<td>378 ± 98</td>
<td>286 ± 68</td>
<td>107 ± 9</td>
<td>100 ± 7</td>
</tr>
</tbody>
</table>

Data are presented Mean ± SD. * represents P < 0.05.
Figure 4.3: Time to complete a 5-km cycling time trial, comparing the mean of three control trials (CON$_{avg}$) to traditional IPC (IPC applied 15-min preceding exercise), IPCx2 (IPC applied 24-h & 15-min preceding exercise) and IPCx3 (IPC applied 48-h, 24-h & 15-min preceding exercise) in A) all participants (n=12), B) participants who were designated as responders (n=7) and C) non-responders (n=5). * represents statistically different from CON$_{avg}$ (P< 0.05).
4.5 Discussion

The present study sought to assess the existence of responders and non-responders to traditional IPC by comparing individual responses to the normal individual variability of a 5-km cycling TT. We also examined whether the benefits induced by the early window of IPC could be enhanced in a dosed, or exaggerated, manner following repeated IPC prior to completing a 5-km cycling time trial. The main findings were that 1) in 7 of 12 participants, traditional IPC improved 5-km TT cycling performance greater than the expected repeated-control CV, and the response or non-response effect was consistently demonstrated; 2) in all participants, and in identified responders, IPC applied in combination of 48-h, 24-h, and acutely preceding (15-min) a 5-km TT cycling performance did not surpass the traditional IPC-mediated exercise performance improvements. These findings suggest that 1) traditional IPC can promote improvements in performance greater than the day-to-day variation around the exercise task; 2) The application of additional IPC doses (at 48-h and/or 24-h) in combination with traditional IPC does not add further performance benefit beyond the effect of a single acute IPC bout alone.

4.5.1 Existence of IPC responders

The current data, when analyzed by group mean, demonstrate traditional IPC to be ineffective for increasing 5-km TT cycling performance. This is consistent with some existing studies (40,131), yet contrasts others who have reported a 1-3 % improvement
in cycling performance (37,50,92). To date, the benefit of IPC on exercise performance remains equivocal in the literature, compounded by evidence that suggests IPC-mediated performance benefits are no more than a placebo effect (101). As previously suggested (91) and demonstrated by our data, inconsistencies in previous findings may well be explained by variability in individual response to IPC, which could misrepresent a true effect when interpreted by group mean. In support of this notion, it is widely recognized that in many other exercise and nutrition related interventions, a large variability in individual response exists (132), and that traditional group intervention-based studies that focus on mean response are limited. Accordingly, an increasing number of investigations are attempting to interpret individual data and classify participants as responders or non-responders to nutrition or exercise-based interventions (115,133,134). Indeed, upon examination of individual participants in the current study, a strong majority (75 %) of participants improved cycling performance. However, it is also evident that within-subject variability is an important consideration when evaluating individual-level response (119). When repeated-control CV was included in the analysis of individual responses, approximately 60% of individuals displayed an ergogenic benefit from traditional IPC that was greater than their repeated-control CV, and these individuals remained verified responders to all IPC interventions >80 % of the time. Thus, the current data provides support that in many individuals, an IPC-mediated performance benefit exists, and merely analyzing data by group means may misrepresent the true effect of IPC.
Although not a specific goal of the current study, it is important to understand individual factors that may explain the responsiveness of IPC. Upon closer examination of our data, we noted a trend toward a significantly higher VO$_2$max in responders (57 ±5ml kg$^{-1}$ min$^{-1}$) vs non-responders (50 ±5ml kg$^{-1}$ min$^{-1}$; p=0.06), and indeed, a higher VO$_2$max was approaching a strong association with being a responder to IPC (r = -0.6; p= 0.06). Importantly, type of responder was not related to sex (r = - 0.1; p=0.9) or variability in performance (5-km TT coefficient of variation, r = -0.03; p=0.8), suggesting against the possibility that this effect is driven by sex or that more highly fit individuals are able to produce a more consistent exercise performance. It is unknown if the physiological characteristics responsible for an augmented VO$_2$max explain the responsiveness to (i.e. IPC oxygen delivery or uptake), or rather if a higher VO$_2$max is simply related to greater cycle training. It is possible that a certain endurance exercise experience is necessary before an individual has the psychological ability to reach the point of effort that is required for IPC to elicit a performance effect. The current study did not record training experience, but it should be considered along with aerobic characteristics when investigating the responsiveness to IPC.

In all performance related interventions, it is important to consider the magnitude of change at which point an alteration is performance is practically meaningful. It has been suggested that, for an athlete, a performance enhancement will alter the chance of winning when it is ~0.3x more than the typical variation between trials (135), and this
threshold value has been termed the smallest worthwhile change (SWC). Hopkins and Patton (130) reported that the SWC in performance time for a top cyclist is ~0.6 % in road time trials; accordingly, verified responders in the current data demonstrated a meaningful improvement in performance from traditional IPC (2±1.6%). As the current study did not investigate top level cyclists, it is perhaps also appropriate to examine the SWC within individual participants. Upon investigation, 5 out of 7 verified responders to traditional IPC presented with a percent change in performance that was greater than their own SWC. Taken on whole, the present findings demonstrate that traditional IPC can also have a practically meaningful benefit to cycling performance.

4.5.2 *Summed effect of the early and late window*

Previous studies have demonstrated that IPC can exert ergogenic effects within both the early and late window. Beaven et al. (89) and Seeger et al. (40) showed comparable benefits to performance, both when IPC is applied either acutely or 24-h preceding running exercise; however, Williams et al. (136) did not demonstrate ergogenic effects when IPC was either applied acutely or 24-h preceding swimming exercise (136). Nonetheless, these studies did not examine the additive effect of combining the early and late windows. The current data demonstrates that compared to traditional IPC, there was no additional improvement in performance when IPC was applied in combination of 48-h, 24-h, and 15-min before a 5-km cycling time trial. This finding was observed in all participants and in verified responders only. This suggests
for the first time that applying IPC within the early and late window is no more effective for enhancing the ergogenic effect of acute IPC. Previous studies have reported that increasing the number of cycles (92), length of cycles (93), or volume of muscle mass subjected to the stimulus (92) is not effective in augmenting the ergogenic effect. Therefore, to date, the current literature suggests that increasing the dose of IPC does not augment the ergogenic effect. The current study also supports the notion that increasing the IPC dose is not detrimental to the IPC effect, as previous research (92) has speculated that implementing too many ischemic cycles may cause hyper-conditioning (137) and negate the ergogenic effect.

As previously mentioned, the benefit of IPC for exercise performance remains a topic of debate, with a prominent theory being that the ergogenic effect of IPC is no more than a placebo effect (101). Ferreira et al. (122) hypothesized that a placebo effect may influence performance, independent of a physiological IPC effect, and may help explain controversial findings about the ergogenic effect of IPC. However, when Ferreira et al. administered three sessions of IPC over two consecutive days, 48-h, 24-h and 30-min prior to repeated sprint swimming trials, performance improvement was significantly greater with IPC (1.17 %) compared with SHAM (0.02 %). It was proposed that IPC may have been more effective than placebo due to the additive effect of the early and late IPC windows. However, the current data suggest that traditional IPC is equally effective as repeated IPC administration for improving
performance. Owing to the design of the present study, which required repeated trials over multiple lab visits, the inclusion of a placebo control was not feasible and thus evaluation of the placebo effect was not an outcome; however, the highly consistent response to IPC suggests it is unlikely that a placebo effect elicits comparable consistency. Further cycling related work from our lab also suggests that even when participants expected IPC treatment to be ergolytic, performance still improved (in press). Finally, during each time trial, only average values of power output, cadence, velocity, and total time to completion were measured.

Physiological variables (i.e. VO$_2$, HR, lactate) were not measured during the time trials and is acknowledged limitation of the current study.

4.6 Conclusion

Following a traditional group mean response analysis, traditional IPC is demonstrated to be ineffective for increasing 5-km cycling performance in experienced cyclists. However, upon examination of individual data, it was found that over half of participants responded to IPC to an extent greater than repeated-control variability, providing support that an IPC-mediated performance benefit exists in some participants. Furthermore, despite known ergogenic effects within both the early and late windows of IPC, it does not appear that repeated and delayed IPC is capable of enhancing ergogenic effect of traditional acute IPC. Finally, these findings have important implications in optimizing IPC-mediated performance benefits.
Chapter 5:
Ischemic Preconditioning: Modulating Pain Sensitivity and its Relationship with an Ergogenic Effect

In Review:

Co-authorship statement:
Joshua Slysz and Jamie Burr conceived and designed the research. Joshua Slysz conducted experiments, analyzed data, and wrote the manuscript.
5.1 Abstract

INTRODUCTION: Ischemic preconditioning (IPC) appears to induce a variety of systemic benefits such as improved exercise performance and reduced perception of post-operative pain. The purpose of this study was to determine if IPC can modulate pain perception among young, healthy individuals, and to determine whether changes in pain sensitivity relate to improvements in exercise performance. METHODS: Thirteen individuals (8 males, 27 ± 6 years, 55 ± 5 ml·kg⁻¹·min⁻¹) underwent two separate cold-water immersion tests: with preceding IPC treatment (CWIIPC) and without (CWI.CON). In each test, the left hand was immersed in water at 1°C, and participants were asked to rate their perceived pain intensity. The time from when pain was first reported to when pain subsided after removal from the water was used to measure total time under pain. In addition, each participant undertook one control and one experimental 5-km cycling time trial. Pearson correlation coefficients were used to compare the association of the ergogenic effect of IPC with the pain sensitivity modulation of IPC. RESULTS: Pain intensity increased over time (p < 0.001) but did not change with IPC (p = 0.9) at any point throughout the test. However, IPC significantly reduced total time under pain compared to control (CWI.CON 141 ± 24s vs CWIIPC 132 ± 20s; p = 0.001). No relationship was found between the change in performance with IPC and the change in time under pain (r = -0.2, p = 0.6) or pain intensity (r = -0.3, p = 0.3) following IPC. CONCLUSION: These findings suggest that IPC can modulate sensitivity to a painful stimulus, but this altered sensitivity is not related to the ergogenic efficacy of IPC.
5.2 Introduction

Ischemic preconditioning (IPC) traditionally involves the exposure of brief periods of circulatory occlusion and reperfusion to a limb that activates protective mechanisms against ischemic-reperfusion injury in local and distant tissues (5,27). The identity of the protective triggers, and mechanisms by which the trigger is conveyed from the distant IPC stimulus to the target tissue, remain incompletely understood; however, previous evidence has implicated an important role of opioids as an endogenous substance released into the bloodstream by preconditioned tissue (18,138,139). It is known that opioid release and activation of opioid receptors at the peripheral, spinal, or supraspinal level can modulate ascending pain information (86). As such, the local or remote release of endogenous opioids via IPC may act to modulate pain sensitivity. Regular exercise is known to reduce sensitivity to painful stimuli in healthy individuals (140) and has been proposed that this is due to the activation of the endogenous opioid system (141,142). In the clinical setting wherein patients have a pre-existing baseline pain, IPC can reduce patient-reported post-operative pain (143,144), but no study has investigated whether IPC can reduce sensitivity to an induced painful stimuli in healthy individuals. Therefore, a primary purpose of this study was to determine if IPC can reduce sensitivity to a cold-water pain in healthy, young individuals.

IPC applied prior to exercise has indeed been shown to improve high-intensity exercise performance, both when brief bouts of circulatory occlusion are applied to
locomotive (37,38) or remote limbs (43,64). The mechanisms underlying these exercise improvements are unknown but have been suggested to be related to both metabolic and vascular alterations (91). Given that endogenous opioid release is known to occur with IPC (18,138,139), and activation of the opioid system is known to reduce pain perception (86), it is reasonable to propose that IPC may reduce the perception of discomfort during a high-intensity exercise task, allowing for an increased effort and performance. Interestingly, circulatory occlusion combined with light exercise reduced anterior knee pain in a clinical population, allowing patients to perform future exercise with higher training loads (145). There appears to be great variability in the exercise performance response to IPC, likely owing to the large between-subject variability evident in the literature (91). This large variability in exercise performance response within and between studies is suggested to be explained by responders and non-responders to IPC treatment (91). It is possible that an individual’s exercise performance response to IPC may be explained by the ability of IPC to modulate one’s own sensitivity to pain or discomfort. Therefore, a secondary purpose of this study was to compare an individual’s IPC-induced change in 5-km cycling time trial performance with the same individual’s IPC induced change in pain sensitivity to a cold stimulus. We hypothesized that IPC would reduce pain sensitivity to a cold-water pain, and that the degree of pain reduction would relate to the individual’s ergogenic response to IPC.
5.3 Methods

5.3.1 Subjects

Thirteen aerobically trained individuals (8 males: 27 ± 7 years, 178 ± 4 cm, 77 ± 7 kg, 55 ± 5 ml·kg⁻¹·min⁻¹, & 5 females: 26 ± 6 years, 168 ± 7 cm, 63 ± 12 kg, 55 ± 7 ml·kg⁻¹·min⁻¹) volunteered to participate in this study, which employed a randomized cross-over design. Recruitment was based on an a priori sample size calculation that accounted for an alpha of 0.05, and a power of 80%. A large effect size (Cohens d = 0.8) was assumed based on the effect observed on RPE with aerobic exercise and pain perception to a similar cold stimulus (146). Participants were non-smokers, with no medical history of chronic disease and were safe to exercise as confirmed through completion of a PARQ⁺ screening questionnaire (110). After being advised of the purpose and potential risks of the study, participants provided written informed consent in accordance with the guidelines of the institutional human research ethics board who approved the experimental protocol and procedures of this study.

5.3.2 Cold-water immersion: experimental design

Each participant performed two separate cold-water immersion tests: i) with preceding IPC administration (CWIIPC), ii) without preceding IPC administration (CWICON). These tests were completed on separate days with at least three days between. The order of the two tests was randomized by the flip of a coin. Participants refrained from alcohol, caffeine, and intensive physical exercise for a least 24 h prior to
both CWI tests. Pain induced by the submergence of the hand in cold water has been used in investigating a wide range of pain management techniques (147) and has an excellent reliability and validity (148).

5.3.3 Cold-water immersion: test protocol

With the participant seated, the left hand was immersed to above the wrist in a bucket of water at 1 degree Celsius for a total of 2 minutes. Each participant was instructed to say “pain” when the cold stimulus first became painful. At the end of the 2 minutes, participants were asked to remove their hand from the cold water and instructed to say “no pain” when the perception of pain had subsided. The time (s) from when the participant first reported pain to when pain was completely gone after removal from the cold stimulus was used to measure total time under pain. Two minutes was set as an upper limit to ensure all participants could complete the entire test without prematurely removing their hand. This allowed for water immersion time to be the same in both tests and total time under pain to be compared. Throughout the 2 minutes of water immersion, each participant was asked to rate the intensity of pain on a 1-5-point Likert rating scale every 15 seconds from “mild” to “excruciating”, as described by the McGill pain questionnaire (149). Participants were naïve to the expected IPC outcome.

Throughout both cold-water immersion tests, beat-to-beat blood pressure was measured from a digit photoplethysmography cuff (Human Non-Invasive Blood Pressure (NIBP); ADInstruments-North America, Colorado, USA) applied to the right
hand. This measurement was also used to measure heart rate. Systolic blood pressure (SBP), Diastolic blood pressure (DBP), heart rate (HR) were measured for 2 minutes at baseline (Pre), during CWI (Mid), and 2 minutes following hand removal from cold water (Post).

5.3.4 Cold-water immersion: IPC protocol

For CWIIPC, an IPC protocol was completed 15 minutes prior to the test. IPC was performed in a seated position using a PTSi automated tourniquet system (Defli Medical Innovations Inc. Vancouver, Canada) with unilateral arterial occlusion of the left arm. The tourniquet cuff was positioned proximally and inflated to a pressure superior to brachial systolic pressure ($\geq 2$ mmHg), allowing complete arterial occlusion. This pressure, called the lowest effective occlusion pressure (LOP), can be detected by the Delfi system for each participant by utilizing a pressure transducer to determine the pressure required to cause the arterial pulsation to disappear (127). LOP was determined in duplicate and the average of these two values was used to set the pressure for circulatory occlusion. Circulatory occlusion lasted 5 minutes and was performed 3 times, each separated by 5 minutes of reperfusion (150). No participant reported undue pain or discomfort during the arm circulatory occlusion.

5.3.5 Exercise performance: experimental design

During a performance familiarization visit, participants completed a maximal oxygen consumption test ($\text{VO}_2\text{max}$) on a cycle ergometer (Velotron Inc, Seattle, USA) to
evaluate aerobic fitness. The test began with a resistance of 100 W and increased continuously (1 watt every 3 seconds) until attainment of VO₂max. Attainment of true physiological max was confirmed for all participants by the presentation of a plateau in VO₂ (increase in ≤50 mL/minute at VO₂ peak and the subsequent data points), and respiratory exchange ratio (RER) ≥ 1.15 (113). Expired gases were measured via indirect calorimetry using a face mask and optical turbine connected to a gas analyser with a sampling line (Cosmed Quark CPET, Rome, Italy). The maximal values were recorded as the highest reading that occurred after the data was smoothed using a rolling 30 s average. Three to five days following the VO₂ max test, participants completed a familiarization 5-km TT to acquaint themselves with the gearing functions of the cycle ergometer and pacing strategies for the 5-km distance. Finish time was not recorded.

Each participant underwent one experimental IPC and one control 5-km cycling time trial. These trials were completed on separate days with a minimum of three days between trials to allow sufficient recovery time. The order of the two tests was randomized by the flip of a coin. Prior to both trials, participants completed a 15-minute warm-up at a power output of 100W on a cycle ergometer. Participants refrained from alcohol, caffeine, and intensive physical exercise for a least 24 h prior to performance testing.
5.3.6 Exercise performance: 5-km cycling time trial protocol

All cycling time trials were completed on the same stationary cycle ergometer as the VO2max test which uses an electromagnetic resistance. Integrated 3D computer software was used to complete a flat, 5-km race, wherein time to completion (s), average and peak power output (Watts), and average revolutions per minute (RPM) were recorded. The system allowed participants to change gears virtually and select their own resistance/RPM to suit their desired pacing strategy. On a computer monitor participants viewed the image of a rider on course (representing the test subject), but were blinded for elapsed time, speed, power output, RPM and specific gearing level, as these variables were removed from the software’s display interface during each trial. Participants were also blinded to the precise distance covered, however, a general guide using a linear scale and representative marker was available for their reference to allow for pacing their work output towards the known endpoint of 5-km. This distance was chosen based on previous research that suggests the most consistent benefit of IPC is for an improvement in time-trial performance in high-intensity exercise tests approaching aerobic capacity (91). In addition, this distance involves less pacing strategy, and was thus chosen to minimize any pacing variability or motivational influences that occur during a prolonged cycling task. Participants were naïve to the expected IPC outcome and received no verbal encouragement throughout any cycling test in order to avoid being influenced by the researchers.
5.3.7 Exercise performance: IPC protocol

Prior to the experimental trial, an IPC protocol was completed 15 minutes prior to the start of the trial. IPC was performed in a seated position using bilateral arterial occlusion of the legs. The tourniquet cuffs were positioned proximally on the thighs and inflated to a pressure that was minimally superior to femoral systolic pressure (≥ 2 mmHg) using the Delfi system as described above. Circulatory occlusion lasted 5 minutes and was performed 3 times, each separated by 5 minutes of reperfusion. No participant reported undue pain or discomfort during the leg circulatory occlusion. Application of cuffs to the legs was chosen to keep the IPC stimulus local to the pain stimulus, as cuff application and cold-water immersion involved the same limb.

5.3.8 Exercise performance: responder & non-responder

In order to identify performance responders and non-responders to IPC, each participant completed two additional control 5-km cycling time trials. These additional control trials were completed at minimum three days after the original control and experimental IPC trail. Each participant’s 3 control trials were used to calculate within-individual variability, represented as the coefficient of variation (CV). Individual performances following IPC (described below) on a 5-km cycling TT were compared to each individual’s CV for the 5-km cycling TT. If a participant’s IPC response was improved to a magnitude greater than his or her own CV, that participant was classified
as a “responder”. If a participant’s response was not outside one’s own CV, that participant was classified as a “non-responder”.

5.3.9 Data analysis and statistics

To determine whether IPC can modulate pain sensitivity, the difference in total time under pain between CWICON and CWIPC was compared using a paired sample t-test. Differences in the intensity of pain throughout the 2 minutes of cold-water immersion were compared between CWICON and CWIPC using a 2 x 8 repeated measures ANOVA with post hoc comparisons if appropriate. Hemodynamic parameters were compared at Pre, Mid, and Post, between CWICON and CWIPC using a 2 x 3 repeated measures ANOVA with post hoc comparisons if appropriate.

The ergogenic effect of IPC on 5-km cycling time trial performance was assessed by calculating percent change between the control and experimental IPC trial. Pearson correlation coefficients were used to quantify and compare the association of the ergogenic effect of IPC with the pain sensitivity modulation of IPC. Specifically, Pearson correlation coefficients were used to relate the percent change in performance with the change in time under pain (s). In addition, area under the curve (AUC) was calculated from the reported pain intensity VAS scores of CWICON & CWIPC. The change in pain intensity (AUC) between the two trials was also related to the ergogenic effect of IPC. Changes in time under pain (s) and pain intensity (AUC) were also related to performance responder types using Pearson correlation coefficients. All Statistical
analyses were conducted using SPSS software (version 25; IBM, Chicago, IL, USA), with differences considered to be statistically significant at P < 0.05. Data is presented as mean ± SD, unless specified otherwise.

5.4 Results

5.4.1 Pain sensitivity modulation

All participants reached the maximum time of 2 minutes in both CWI\textsubscript{CON} and CWI\textsubscript{IPC}. There was a significant reduction in time under pain after IPC (132 ± 20 s) compared to control (141 ± 24 s; Cohen’s d= 2.5; p = 0.001; Figure 5.1). Pain intensity increased with progressive time in both CWI tests (p< 0.001), however, this rate of increase was not altered by IPC (p= 0.96), nor did IPC have an effect on pain intensity at any point throughout the test (p= 0.16; Figure 5.2). SBP, DBP, MAP, and HR increased with progressive time in both CWI tests (p < 0.05), however, these responses were not different following IPC (p > 0.05), nor did IPC influence SBP, DBP, MAP, or HR at any point throughout the test (p >0.05; Table 5.1).
Figure 5.1: Time (s) under pain during a cold-water immersion test under normal conditions (CWI\textsuperscript{CON}) and following IPC treatment (CWI\textsuperscript{IPC}). The time under pain represented the total time (s) from when the participant first reported pain after introduction of the cold stimulus to when the pain was completely gone after removal of the cold stimulus. Data is represented as mean ± SD, and * represents a statistically significant difference (P < 0.05).

Figure 5.2: Ratings of pain intensity as reported on a 1-5-point Likert scale every 15 seconds during a cold-water immersion test in control (CWI\textsuperscript{CON}) and experimental IPC (CWI\textsuperscript{IPC}) conditions. Data is represented as mean ± SD.
5.4.2 Exercise performance vs pain sensitivity modulation

Mean performance improved with IPC by 0.8 ± 2 % over the 5-km TT, however, as a result of large inter-individual differences driving variability, this mean performance improvement did not reach statistical significance (497 ± 39 s vs. 494 ± 43 s, respectively, Cohen’s d=0.65, p= 0.3,). There were no significant associations between the percent change in performance with IPC and the change in time under pain with IPC (r = -0.2, p = 0.6, Figure 5.3A), or the change in pain intensity (AUC) with IPC (r = -0.3, p = 0.3, Figure 5.3B).

### Table 5.1: Hemodynamic parameters measured throughout a cold-water immersion test without (CWI\textsubscript{CON}) and with (CWI\textsubscript{IPC}) preceding IPC administration. Data is represented as mean ± SD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre (2 mins)</th>
<th>During (average)</th>
<th>Post (2 mins)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>CWI\textsubscript{CON} 123 ± 7</td>
<td>148 ± 14</td>
<td>135 ± 15</td>
<td>Group = 0.8 Time &lt; 0.001 Interaction = 0.5</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 123 ± 11</td>
<td>148 ± 18</td>
<td>132 ± 14</td>
<td>Time &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{CON} 126 ± 12</td>
<td>149 ± 16</td>
<td>136 ± 15</td>
<td>Time &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 125 ± 15</td>
<td>148 ± 17</td>
<td>135 ± 15</td>
<td>Time &lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>CWI\textsubscript{CON} 66 ± 11</td>
<td>82 ± 11</td>
<td>72 ± 11</td>
<td>Group = 0.9 Time &lt; 0.001 Interaction = 0.6</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 65 ± 11</td>
<td>83 ± 12</td>
<td>72 ± 12</td>
<td>Group = 0.9 Time &lt; 0.001 Interaction = 0.6</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{CON} 85 ± 10</td>
<td>104 ± 12</td>
<td>93 ± 12</td>
<td>Group = 0.9 Time &lt; 0.001 Interaction = 0.6</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 84 ± 11</td>
<td>105 ± 14</td>
<td>92 ± 13</td>
<td>Group = 0.9 Time &lt; 0.001 Interaction = 0.6</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>CWI\textsubscript{CON} 55 ± 12</td>
<td>95 ± 11</td>
<td>65 ± 10</td>
<td>Group = 0.6 Time &lt; 0.001 Interaction = 0.9</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 57 ± 8</td>
<td>76 ± 12</td>
<td>66 ± 9</td>
<td>Group = 0.6 Time &lt; 0.001 Interaction = 0.9</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>CWI\textsubscript{CON} 65 ± 12</td>
<td>75 ± 11</td>
<td>65 ± 10</td>
<td>Group = 0.6 Time &lt; 0.001 Interaction = 0.9</td>
</tr>
<tr>
<td></td>
<td>CWI\textsubscript{IPC} 67 ± 8</td>
<td>76 ± 12</td>
<td>66 ± 9</td>
<td>Group = 0.6 Time &lt; 0.001 Interaction = 0.9</td>
</tr>
</tbody>
</table>
Figure 5.3: Correlation between the change in 5-km TT performance with IPC with: A) the change in time under pain (s) after IPC (time under pain represented total time from when the participant first reported pain after introduction of the cold stimulus to when the pain was completely gone after removal of the cold stimulus), and B) the change in pain intensity area under the curve with IPC; area under the curve was calculated from the reported pain intensity Likert scores from a cold-water immersion test.

5.4.3 Performance responder type vs pain sensitivity modulation

If a performance improvement was only considered true when a participant’s individual IPC response exceeded one’s own percent CV from repeated controls (group average CV: $1 \pm 0.6 \%$) then 6 (46 %) would be classed as verified responders and 7 (54 %) as non-responders. There were no significant correlations found between performance responder type and the change in time under pain with IPC ($r = -0.01$, $p = 0.9$), or the change in pain intensity (AUC) with IPC ($r = 0.1$, $p = 0.4$).
5.5 Discussion

The present study sought to investigate whether IPC could reduce pain sensitivity to an introduced painful stimulus in healthy, young individuals, while also comparing IPC-induced changes in pain sensitivity to IPC-induced changes in 5-km cycling time trial performance. The main findings were that 1) IPC administration prior to a cold-water immersion test decreased total time under pain; however, it did not change perception of pain intensity during the cold stimulus, 2) IPC-induced changes in pain sensitivity were not related to IPC-induced changes in 5-km time trial performance. These findings provide evidence that IPC reduces external pain sensitivity; however, an individual's change in exercise performance after IPC is not explained by the IPC effect on external pain sensitivity.

5.5.1 IPC pain modulation

To date, no work has investigated the use of an IPC protocol on reducing sensitivity to a purposely introduced painful stimulus in healthy individuals. Previous literature, however, has investigated the analgesic effect of IPC in a clinical setting, indicating that postoperative pain intensity (143,144), morphine use (143), and mean hospital stay (144) can be reduced in patients who undergo IPC before a surgical procedure. In like manner, the current study found that IPC can modulate pain sensitivity by reducing total time under pain; in contrast, however, absolute pain intensity was not affected. It should be pointed-out that previous clinical research
(143,144) evaluated pain intensity in the recovery from a painful stimulus, while the current study evaluated pain intensity during the administration of the painful stimulus and not after its removal. Therefore, it remains possible that IPC can reduce pain intensity during withdrawal of a painful stimulus in young, healthy individuals, and should be considered by future research as it may have implications for sports that include intermittent rest and recovery.

Previous research suggests that the mechanisms by which IPC modulates pain sensitivity is related to the release of endogenous opioids (138). It is known that an ascending painful stimulus from a remote body area exerts descending analgesic effects on the perception of another painful stimulus (151), a phenomenon (known as “diffuse noxious inhibitory control”) that is likely to be mediated by endogenous opioids (152). Interestingly, Pertovaara et al. (153) showed that ischemic pain induced by cuff application to the arm combined with handgrip exercise reduced cold sensitivity, which was lost with administration of an opioid blockade. Thus, it may specifically be the ischemic pain imposed by cuff application that is responsible for opioid release and pain sensitivity modulation with IPC.

In the current study, it is unknown how IPC reduced time under pain to such a large effect (Cohen’s d = 2.4), while having no measurable effect on pain intensity. The data revealed that the reduction in total time under pain was mainly driven by a reduction in the time it took for pain to subside after removal from the painful stimulus.
Therefore, it is possible that ischemic pain and the degree of opioid release from 5
minutes of unilateral IPC was insufficient to decrease the intensity of the cold pain;
thereby explaining the presence of an effect only when the painful stimulus was
removed. Exercise has also shown to be effective in reducing pain intensity (140) via
endogenous opioids (154–157), and indeed a certain exercise intensity and duration is
needed before an analgesic effect (158). As such, an augmented IPC stimulus (159)
may be necessary to elicit an adequate response and observe a reduction in pain
intensity during the painful stimulus and this may alter the observed relationship
between pain sensitivity and exercise performance.

5.5.2 IPC ergogenic effect vs IPC pain modulation

Certain types of high intensity exercise are perceived as painful. Indeed,
reproducible relationships between objective measures of exercise intensity and
subjective assessment of leg muscle pain intensity during cycle ergometry have been
reported (160). Therefore, if IPC can decrease an individual's perception of this pain,
his/her performance response following IPC may be explained by a decrease in muscle
pain sensitivity during an intense exercise task, allowing for increased effort and
improved performance. However, in the current study, IPC-mediated reductions in pain
sensitivity were not related to IPC-mediated improvements in performance. This finding
suggests that a reduction in pain sensitivity did not explain an individual's performance
response following IPC. In addition, a reduction in pain sensitivity did not relate to a
verified IPC performance responder, providing evidence that reductions in pain sensitivity do not explain the ergogenic effect of IPC.

The aforementioned findings were unexpected given that previous literature demonstrates an improved exercise performance after IPC administration without corresponding improvements in aerobic metabolism (38,73,82,159). It is still possible that IPC improves exercise performance through perceptual modulation, as the mechanism by which IPC modulates an individual's perception to cold pain may not equate to how IPC modulates his/her perception of exercise. It is important to note that the pain of a cold-pressor test is driven by a single external stimulus, whereas the discomfort of intense exercise is internal and dependent on multiple signals across organs. Along these lines, it has been alternatively theorized that IPC may improve performance by modulating sensitivity to fatigue rather than pain (38,82). Future research should continue to investigate the possibility that IPC-induced improvements in exercise performance are related to perceptual changes during the exercise task.

5.6 Limitations

Certain limitations of this study need to be considered. It is possible that the reactive hyperemic response after cuff deflation with IPC confounds cold pain perception. As such, the reduction in pain recovery time with IPC may be explained by increased blood flow to the hand. However, pain intensity was not affected by IPC, which would be unexpected if an increase in blood flow and consequent increase in limb
temperature occurred. Regardless, future research should consider applying IPC to a limb remote from the area being subject to pain in order to avoid local reactive hyperemic effect. The limited resolution of the 0-5 point Likert scale could be a possible reason for the non-significant change in pain intensity following IPC. However, the use of a high-resolution scale (i.e. visual analog scale) was not feasible as both hands of the participant were involved in the testing and not available to make a handwritten mark.

5.7 Conclusion

In a group of young, healthy subjects, IPC reduces pain sensitivity during a painful cold stimulus. However, the current study does not support a strong relationship between pain sensitivity modulation and the ergogenic effect of IPC even when considering those “responders” for whom IPC appears most effective.
Chapter 6
Impact of Eight Weeks of Repeated Ischemic Preconditioning on Running Performance

Presented as published:


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Co-authorship statement:

Joshua Slysz and Jamie Burr conceived and designed the research. Joshua Slysz conducted experiments, analyzed data, and wrote the manuscript.
6.1 Abstract

Purpose: To examine if repeated exposure to IPC treatment prior to training sessions improves oxygen uptake and 1-km running performance in highly trained middle-distance runners. Methods: Fourteen highly trained endurance runners (11 male/3 female, 19±2 years, 64±5 ml·kg\(^{-1}\)·min\(^{-1}\)) completed a baseline maximal oxygen consumption (VO\(_{2\max}\)) test and 1-km running performance test before random assignment to an IPC or control group. Both groups were prescribed identical endurance training over an 8-week varsity season; however, the IPC group performed an IPC protocol (5 minutes ischemia, repeated 3 times, each separated by 5 minutes reperfusion) before every training session. After 8 weeks of training, participants completed a follow-up VO\(_{2\max}\) test and 1-km time trial. Results: VO\(_{2\max}\) did not increase from baseline in either group following the 8-week training bout (P=0.2), and neither group varied more than the other (ΔVO\(_{2\max}\)=IPC 0.6±2 ml·kg\(^{-1}\)·min\(^{-1}\); control 1.5±2 ml·kg\(^{-1}\)·min\(^{-1}\), P=0.6) or beyond typical measurement error. The IPC decreased 1-km time trial time by 0.4% (0.5±2 s), while the control group decreased by 1% (1.5±3 s), but neither change was significant compared to baseline (P=0.2). There was also no difference in time trial improvement between IPC and control (P=0.6). However, there was a trend towards IPC significantly improving running economy at low intensity (P=0.057). Conclusion: Our data suggest that over a normal 8-week season in a population of highly trained middle-distance runners there is no benefit of undergoing
chronic, repeated IPC treatments before training for augmenting maximal aerobic power or 1-km performance time.

6.2 Introduction

Competitive athletes seek to optimize a training framework that stimulates progressive adaptation and performance. Due to diminishing returns on traditional training, athletes often look for additional preparatory strategies or techniques in an attempt to gain a competitive advantage; some of which are used to augment training effects and recovery over time, while others are applied just prior to competition to provide a more immediate advantage. A recent strategy for acutely improving performance is ischemic preconditioning (IPC) (37,43). IPC is characterized by the application of brief periods of circulatory occlusion and reperfusion of a limb in the minutes to hours preceding exercise (1). As a method that is easily administered, non-invasive, and inexpensive, it represents an attractive ergogenic aid for athletes to augment performance.

The most consistently observed athletic benefit of acute IPC treatment is an improvement in time-trial performance for events lasting >75 s (91). While IPC is more likely to be ergogenic during endurance exercise, the mechanisms that underlie the ergogenic effects of IPC are unclear. Recently, Griffin et al. (70) observed an improved critical power following IPC and suggested that IPC has the potential to improve endurance performance by enhancing capacity in the severe intensity exercise domain.
Acute application of IPC improves muscular endurance at severe-intensity, as evidenced by improved skeletal muscle oxygenation (67,68); while short term (7-10 days) (161) and long term (8 weeks) (162) exposure to repeated IPC sustains this enhanced skeletal muscle oxidative capacity beyond the reported acute late phase of protection following IPC (72 h) (58). In addition, it is known that IPC can improve recovery from muscle damaging exercise (163,164), and it is therefore possible that IPC would allow athletes to maximize outputs during each training session. As such, repeated exposure to IPC should permit for an enhanced high-intensity endurance performance over time. However, to date, studies investigating the effectiveness of repeated IPC for improving performance remain limited and conflicting (165,166).

High performance research completed within an athlete’s complex training environment is of practical value for both coach and athlete (167). Evidence suggests that IPC may be efficacious as an ergogenic aid to gain a competitive edge, however, current literature lacks a proper evaluation of IPC as a tool that can be practically applied over the course of an athlete’s training program. Thus, the purpose of this study was to determine the impact of consistently, and repeatedly undergoing treatment of IPC within an athlete’s training block for improving high-intensity endurance performance over time. Specifically, we aimed to investigate the changes in 1-km time trial running performance and maximal oxygen consumption, in highly trained middle-distance runners who employed IPC treatment before training sessions for 8 weeks. It
was hypothesized that athletes who underwent IPC treatment would have a greater maximal oxygen consumption and an improved 1-km time trial performance after 8 weeks of training compared with similar athletes who did not undergo IPC treatment.

6.3 Methods

6.3.1 Subjects

Sixteen highly trained middle-distance runners were recruited from the most successful Varsity Track & Field team in the country. For all participants, 1-km was a competitive distance with which they were familiar. Fourteen participants completed the study, and two participants withdrew due to injuries unrelated to the study protocol. At the time of the study, all runners were involved in regular training (10-15 h/week). Their personal best times for the 1000 m are shown in Table 6.1. All participants had a high maximum oxygen consumption (\(\text{VO}_2\text{max} \), 65 ± 6 ml·kg\(^{-1}\)·min\(^{-1}\)). The study was approved by the University ethics committee and conformed to the Declaration of Helsinki, with all participants providing written informed consent prior to enrollment.

Table 6.1: Personal best running times for the 1000 m run of the 15 athletes in the study.

<table>
<thead>
<tr>
<th>Athlete</th>
<th>Experimental Personal best time (s)</th>
<th>Athlete</th>
<th>Control Personal best time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150.37</td>
<td>1</td>
<td>188.22</td>
</tr>
<tr>
<td>2</td>
<td>150.07</td>
<td>2</td>
<td>148.66</td>
</tr>
<tr>
<td>3</td>
<td>190.00</td>
<td>3</td>
<td>154.91</td>
</tr>
<tr>
<td>4</td>
<td>151.25</td>
<td>4</td>
<td>186.43</td>
</tr>
<tr>
<td>5</td>
<td>156.11</td>
<td>5</td>
<td>159.70</td>
</tr>
<tr>
<td>6</td>
<td>155.03</td>
<td>6</td>
<td>181.11</td>
</tr>
<tr>
<td>7</td>
<td>161.14</td>
<td>7</td>
<td>145.09</td>
</tr>
</tbody>
</table>
6.3.2 *Research design*

Participants reported to the laboratory to complete a baseline \( \dot{V}O_2\text{max} \) test. At least 48 hours later, but within one week, all participants then performed a 1-km running time trial on a 200 m indoor polytan track surface. Stratified randomization by sex and coin flip was used to assign athletes to either the IPC treatment (n=7, 6 male/1 female, 18 ± 1 y, 67 ± 3 kg, 178 ± 3 cm) or control (n= 7, 5 male/2 female, 19 ± 2 y, 67 ± 10 kg, 181 ± 12 cm) group. Both groups were prescribed identical training over the next 8 weeks; however, the IPC group performed IPC treatments before training. The groups were initially matched for sex, but due to dropout, 1 female in the IPC group and 1 male in the control group did not complete the study. After 8 weeks of training, each participant underwent a follow-up time trial and \( \dot{V}O_2\text{max} \) test at least 48 hours, but at most 1 week following the last IPC intervention. Participants refrained from alcohol, caffeine and strenuous exercise 24 hours before all baseline and follow-up performance testing.

6.3.3 *Experimental measurements*

A continuous incremental test on a motorized treadmill (Pulsar 4.0; h/p/cosmos, Willich, Germany) was used to assess maximal oxygen consumption (\( \dot{V}O_2\text{max} \)). The test commenced after a 5-minute self-paced warm-up ranging between 4 and 6 mph. Continuous 2-minute stages were performed (0.5 mph and 1 % grade increment per stage) starting at 6 mph for females and 9 mph for males. During the test, breath by-
breath expired gases were monitored using open circuit spirometry (COSMED Quark CPET, Rome, Italy) and VO2 (ml kg\(^{-1}\) min\(^{-1}\)), ventilation (VE, L min\(^{-1}\)), and RER were averaged over the last 30s of breath-by-breath data of each stage. This VO2 average at each stage was used to investigate the oxygen cost at each running velocity over this time (running economy). Achievement of VO2\(_{\text{max}}\) was considered as the attainment of a plateau in oxygen uptake (< 150 mL/min increase) despite increasing workload, a rating of perceived exertion ≥ 19-20, and an RER ≥ 1.15. The maximal values were represented as the highest value to occur after the data was smoothed using a 30s rolling average.

Before each 1-km time trial, participants performed a standard 15-minute warm-up period that included low-intensity running, several acceleration runs and stretching, as per individual race preparatory strategy. Time trials were performed individually, with athletes wearing running spikes typically used during competition. Participants were advised to use a normal race strategy and were verbally encouraged by the researcher and their coach to perform to maximum effort during the trial. Performance times were recorded every 200 m by the same researcher using a hand-held chronometer (Lowell YT0401, China), and athletes were informed of their single lap times at 200 m and 400 m but were blinded to their last 3 lap times. This was to ensure that athletes ran by feel, as opposed to locking in on a predetermined pace, which may have prevented potential
improvement. Baseline and follow-up time trials were scheduled around the same time of day between 15:00 h - 18:00 h.

6.3.4 Training Intervention

During the time of the study, athletes were in a general preparation training phase and did not participate in any competitive event. All participants underwent the same training involving 3 organized high-intensity group sessions and 3 additional lower-intensity individual running sessions per week, for 8 weeks. Each high-intensity group session involved a total of 40 minutes at an easy intensity (~ 4.7 min/km), 10 minutes at a tempo intensity (~ 3.3 min/km), and 3 minutes at 1-km race pace (~ 2.5 min/km). Each week, the three high-intensity sessions totalled ~ 160 minutes and ~40 km of running. The three lower-intensity individual running sessions per week included two sessions of 45 minutes and one session of 70 minutes at a low-intensity pace (~ 4.7 min/km) that totalled ~ 160 minutes and ~ 35km of running. Over the course of the 8-week study, training was periodized by gradually exposing athletes to their specific race pace (1000 m) in preparation for the upcoming indoor season set to begin soon after the conclusion of the 8-week study. This training was designed and implemented by the coach. Participants in the IPC group arrived 40 minutes before the start of all high-intensity group sessions to undergo an IPC treatment. Compliance with training session participation over the 8 weeks was 92%-100%.
Ischemic preconditioning was performed in a sitting position using unilateral arterial occlusion of a leg. Occlusion was accomplished using a PTSi automated tourniquet system (Defli Medical Innovations Inc. Vancouver, Canada). Unilateral IPC has been proposed to induce a systemic preconditioning effect (5), and past research using a unilateral IPC model has shown to improve performance in whole body exercise such as 100 m swim time (43) and 1-km rowing time (41). The occlusion cuffs were positioned proximally around the right thigh and inflated above systolic pressure, ensuring complete arterial occlusion. Specifically, we employed the lowest effective occlusion pressure (LOP), which can be detected by the Delfi system for each participant by utilizing a probe to detect the pressure required to cause the distal pulse to disappear (127). LOP was determined in duplicate and the average of these two values was used to set the pressure for circulatory occlusion. Circulatory occlusion lasted 5 minutes and was performed 3 times, each separated by 5 minutes of reperfusion. No participant complained of undue pain or discomfort during the periods of leg circulatory occlusion.

6.3.5 Statistical analysis

The primary outcomes were performance time to complete a 1-km time trial and maximal oxygen consumption during a continuous incremental running test to exhaustion. These variables were analyzed using a 2x2 repeated measures ANOVA (2 level: IPC and Control) and time (2 levels: pre and post 8-week intervention). Similarly,
a 2-factor general linear model was used to analyze the submaximal variables of $\dot{V}O_2$, VE, and RER during the first three stages of the continuous incremental running test to exhaustion. Significant effects were examined using the least significant difference method for pairwise multiple comparisons. For all statistical tests, a 2-tailed comparison was used. Data are reported as mean ± SD unless stated otherwise, and statistical significance was set at $P \leq 0.05$. All statistical analyses were performed using SPSS 25.0 software (SPSS, Chicago, IL).

6.4 Results

6.4.1 1-km time trial

As a whole, the athletes appeared to be marginally faster by 0.7 % (-1.1 ± 0.7 s, 95% CI [-2.5, 0.2] Cohen’s $d= 0.34$) following 8 weeks of training, but this change in performance on the 1-km time trial did not reach significance as a main effect ($P= 0.2$). There was also no significant difference in the magnitude of improvement on the time trial performance between IPC (0.4 %, -0.5 ± 2 s, 95% CI [-2.5, 1.4], Cohen’s $d= 0.2$) and control (1 %, -1.5 ± 3 s, 95% CI [-3.7, 0.23], Cohen’s $d= 0.5$) groups ($P= 0.4$, Figure 6.1).
6.4.2 Maximal aerobic performance

There was no detectable increase in VO$_2$max in either group following the 8-week training block (P= 0.2, Cohen’s d= 0.4), and neither the IPC group (ΔVO$_2$max= 0.6 ± 2 ml/kg/min, 95% CI [-0.7, 1.7], Cohen’s d= 0.4) or Control group (1.5 ± 2 ml·kg$^{-1}$·min$^{-1}$, 95% CI [0.24, 2.7], Cohen’s d= 0.6) increased or decreased more than the other (P=0.6, Figure 6.2). Table 6.2 shows there were no significant changes across time and between groups in submaximal VO$_2$, VE, and RER throughout the continuous incremental test. However, there was a trend found toward a significant decrease (P= 0.057; Cohen’s d=0.55) in VO$_2$ at the lowest-intensity submaximal exercise stage.
Figure 6.2: Individual and mean (SD) VO₂ max performance before and after 8 weeks of IPC treatment before training three times per week in young highly trained middle-distance runners. *denotes a significant main effect of group (P=0.03)
Table 6.2: Oxygen Consumption (VO₂), Ventilation (VE) and Respiratory Exchange Ratio (RER) collected during the first three stages of an incremental exercise test before and after 8 weeks of training with or without regular IPC treatment.

<table>
<thead>
<tr>
<th></th>
<th>Stage 1</th>
<th>P Value</th>
<th>Stage 2</th>
<th>P Value</th>
<th>Stage 3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>VO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IPC</td>
<td>47.1 ± 2</td>
<td>44.4 ± 3</td>
<td>50.7 ± 3</td>
<td>49.0 ± 3</td>
<td>56.2 ± 3</td>
<td>55.5 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>42.1 ± 4</td>
<td>42.0 ± 4</td>
<td>48.3 ± 4</td>
<td>48.8 ± 4</td>
<td>54.6 ± 5</td>
<td>54.2 ± 4</td>
</tr>
</tbody>
</table>

IPC:
- Time: 0.1
- Group: 0.09
- Time X Group: 0.06

Control:
- Time: 0.7
- Group: 0.7
- Time X Group: 0.4

VE

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<tbody>
<tr>
<td>IPC</td>
<td>88.6 ± 8</td>
<td>86.1 ± 9</td>
<td>103 ± 5</td>
<td>99 ± 4</td>
<td>122 ± 9</td>
</tr>
<tr>
<td>Control</td>
<td>83.3 ± 16</td>
<td>80.4 ± 13</td>
<td>94 ± 17</td>
<td>96 ± 16</td>
<td>120 ± 18</td>
</tr>
</tbody>
</table>

IPC:
- Time: 0.9
- Group: 0.1
- Time X Group: 0.8

Control:
- Time: 0.7
- Group: 0.8
- Time X Group: 0.2

RER

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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IPC</td>
<td>0.88 ± 0.1</td>
<td>0.86 ± 0.1</td>
<td>0.88 ± 0.1</td>
<td>0.86 ± 0.1</td>
<td>0.92 ± 0.1</td>
<td>0.89 ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>0.87 ± 0.1</td>
<td>0.86 ± 0.1</td>
<td>0.87 ± 0.1</td>
<td>0.86 ± 0.1</td>
<td>0.93 ± 0.1</td>
<td>0.90 ± 0.1</td>
</tr>
</tbody>
</table>

IPC:
- Time: 0.6
- Group: 0.3
- Time X Group: 0.9

Control:
- Time: 0.7
- Group: 0.1
- Time X Group: 0.3

IPC:
- Time: 0.5
- Group: 0.2
- Time X Group: 0.8
6.5 Discussion

This exploratory trial examined the impact of undergoing consistent, repeated use of IPC before training for improving maximal oxygen consumption and 1-km running performance in highly trained middle-distance runners. The main finding of this study was that eight weeks of IPC treatment before three high-intensity training sessions per week did not improve maximal oxygen consumption nor 1-km time trial performance more effectively than a control group that did not undergo IPC treatment. Therefore, our data suggest that over the course of a typical 8-week season there is no apparent benefit of undergoing repeated IPC treatment before training for improving maximal oxygen consumption or running performance over 1-km in a population of highly trained middle-distance runners.

6.5.1 Maximal aerobic performance

While the current data demonstrate repeated IPC to be ineffective for increasing \( \text{VO}_2\text{max} \) after 8 weeks, previous research has supported the notion of a sustained aerobic adaptation as a result of using IPC. Short-term (7-9 consecutive days) IPC administration has shown to increase skeletal muscle oxidative capacity (161), enhanced microvascular oxygenated blood flow (161), and improved vascular conductance (168). These adaptations have further been shown to be maintained over the long term (8 weeks) when IPC is applied consistently (162). Despite these tissue-level aerobic adaptations from repeated IPC, data supporting a translation to improved
whole-body VO$_2$max remains conflicted. No change in VO$_2$max was observed after 9 consecutive days of IPC (165) but Lindsay et al. (166) observed a very large 9.5 % improvement in VO$_2$max after 7 consecutive days of IPC, and 12.8 % after 7 additional days of no IPC. These improvements in VO$_2$max are substantially greater than changes in VO$_2$max after a singular bout of IPC (3%) as previously reported by de Groot et al. (37). Our data suggest that long-term exposure to repeated IPC in well-trained runners either does not cause, or does not maintain, these previously reported effects of short-term repeated IPC on VO$_2$max. Discrepancies in the populations used between studies should be recognized as the current study used well-trained athletes, while Lindsay et al. (166) recruited recreationally active participants. It is known that when untrained individuals initiate a training program VO$_2$max improves rapidly, but as an individual becomes increasingly well-trained, the returns in VO$_2$max diminish as genetic limits are approached (169). In addition, the current study did not apply IPC on consecutive days as did Lindsay et al. (166), but rather used three non-consecutive times per week. The optimal methodology (frequency, intensity, and duration) for implementing repeated IPC is unknown, and differences between previous studies and the current study could be explained by different methodology of application. The determinants of VO$_2$max are complex, but it is generally accepted that exercise in which a large muscle mass is recruited stresses both the central and peripheral limitations of oxygen delivery and uptake (170). Jones et al. (168) observed an improved vascular conductance after 7 consecutive days of repeated IPC, and after 8 weeks of 3 non-consecutive days per
week, suggesting daily IPC episodes may not be necessary for this effect. If an IPC-mediated improvement in vascular conductance is the mechanism leading to changes in VO$_2$max, it is unknown why the current study did not observe a such a change. Interestingly, 4 weeks of applying blood flow restriction after sprint interval training has recently been shown to increase VO$_2$max (171,172), suggesting an augmented metabolic stress may be important in long-term aerobic adaptation. Mitchell et al. (172), did not observe any peripheral adaptations following 4 weeks of repeated IPC after sprint training and suggested that the increase in VO$_2$max is perhaps more likely attributed to central adaptations, i.e., increase cardiac output. It is possible that frequency and/or intensity of the IPC stimulus needs to enough to elicit central adaptations and increases in VO$_2$max.

6.5.2 1-km time trial

Previous studies have investigated running performance after a singular bout of IPC, reporting an increase in 5-km performance on a laboratory treadmill, but not on an athletic track (39,131). Existing literature investigating the effects of repeated IPC on athletic performance has demonstrated that 4 weeks of applying IPC after sprint interval training has no effect on 15-km running performance (171) or cycling critical power (173). In agreement, our findings demonstrate no change in running performance, using a 1-km time trial, following 8 weeks of repeated IPC.
Previous research has shown that during events as short as 2 minutes, exercise intensity will be above 90% of VO$_2$max and this intensity may be even higher for longer middle-distance events (174). Since the average time for the 1-km time-trial in the current study was $169 \pm 14$ s, athletes were very likely close to VO$_2$max throughout the test. As we observed no change in VO$_2$max, it is perhaps not surprising that there was no change in 1-km time trial performance. Finally, it is worth recognizing that treatment-based differences may have occurred in the IPC group if training were done by individual feel/effort and not as a training group, as the IPC group may have opted to train differently (i.e. harder/longer) if IPC allowed for a greater output of each session.

During submaximal exercise there was a trend toward a decrease in VO$_2$ at the lowest-intensity stage of the graded exercise test in the IPC group. A single bout of IPC has been shown previously to have no effect on submaximal VO$_2$ (88), yet Jefferies et al. (161) reported a decrease in resting muscle metabolism and improved efficiency when performing submaximal (40% 1 RM) plantar flexion exercise in young, active males after 7 consecutive days of IPC. The effect of repeated IPC on running economy has not been previously investigated. An increased running economy could facilitate improved running performance, as it is known that in highly trained distance runners with similarly high VO$_2$max, running economy is a better predictor of performance than VO$_2$max (175). As previously mentioned, athletes in the current study were most likely at their VO$_2$max throughout most of the 1-km performance trial, thus any improvement
in submaximal running economy would not be reflected in performance. However, these improvements in low-intensity running economy may benefit longer-distance endurance athletes whose typical race pace is further from VO\textsubscript{2}max. Interestingly, running economy was improved at an intensity of 6 and 9 mph for females and males, respectively; a pace that is much lower than typical race pace for middle distance events. At present, changes in submaximal economy after chronic repeated use in training remains speculative and warrants further investigation.

It must be recognized that owing to a limited number of potential participants and small change in performance this investigation had a low statistical power, which inflates the possibility of type II error. However, even in the presence of a type II error, the observed effect was not a change that was meaningful in the context of highly trained middle-distance runners. Repeated IPC treatment resulted in a 0.4% performance improvement after 8 weeks. In all performance related interventions, it is important to consider the magnitude of change at which point an alteration is performance is practically meaningful. It has been suggested that, for an athlete, a performance enhancement will only alter the chance of winning when it is greater than the typical variation between trials. Malcata and Hopkins (176) reported a 1% typical variation in highly trained runners for distances < 3-km. As such, a 0.4% improvement from 8 weeks of repeated IPC treatment does not have a practically meaningful benefit to 1-km time trial performance in highly trained distance runners. Therefore, even with a
higher probability of a type II error, it does not weaken our conclusion of repeated IPC being ineffective for improving 1-km time trial performance after 8 weeks.

### 6.6 Limitations

As with any study, there are a number of limitations to this work that must be recognized. We acknowledge the limitation of the unilateral IPC model, as it could be argued that bilateral IPC or augmented IPC (see study 1) is needed to provide a sufficient stimulus in order to elicit a physiologic adaptation. The majority of literature suggests no clear relationship between the effects of IPC on performance and amount of muscle mass subjected to ischemia (91), as time trial performance has been improved by applying IPC to the arm (41,43) or leg (38,39,64). Additionally, the cytoprotective effects of IPC have been shown to be similar when occlusion was completed three times in the arms and legs (58), and the number and duration of cycles rather than the tissue mass exposed to IPC determines the efficacy of protection (177). However, recent literature specifically examining IPC dosing suggests that bi-lateral occlusion, but not a greater number of IPC cycles, may provide benefit (92). It is noted, however, that this work was performed in cyclists, not runners and the transfer across sports remains unclear.

Comprehensive individual athlete monitoring was not completed by the research team above and beyond the normal athlete-coach interactions and, thus, details on changes in external factors such as nutrition, sleep, or mood states etc. over the 8
weeks are not known. While this was a necessity for the ecological validity and feasibility of this study it is important to note that the observed non-effect of repeated IPC treatment could have been caused by an uncontrolled variable. Particularly, athletes did not restrict caffeine throughout the 8 weeks due to the impracticality of its exclusion during athletic training. As caffeine has been shown to abolish the cytoprotective effects of IPC (94), it is possible that the IPC effect could have been blunted throughout training. Finally, the inclusion of a sham control for the non-experimental group was omitted, due to limited number of available athletes. As such, it is possible that a placebo effect could have occurred, if participants believed the treatment would help. However, the absence of an IPC effect indicated a lack of placebo effect.

6.7 Conclusion

In a group of highly trained middle-distance endurance runners, 8 weeks of consistent, repeated exposure to IPC prior to training did not improve VO₂max or running performance over 1-km. These findings suggest that the application of IPC as an ergogenic strategy to enhance the training effect and improve high-intensity running performance over a relatively short distance may not be useful in a typical team setting. However, whether this technique could enhance the training effect over time to be ergogenically useful for submaximal running economy or another outcome remains unknown.
Chapter 7– Integrative Discussion

7.1 Summary of findings

The purpose of this thesis was to develop an understanding of how to best exploit IPC-mediated exercise performance improvements, facilitating optimal application in athletics. To address this purpose, three specific research aims were implemented, and four independent experimental studies were conducted.

The first aim of this thesis investigated new variations of IPC administration in an effort to enhance the IPC stimulus and augment the ergogenic effect. In study 1 (Chapter 3), the IPC stimulus was enhanced by amplifying the metabolic stimulus through combining IPC with either active walking or passive electrical muscle stimulation. Here, it was highlighted that amplification of the metabolic stimulus is effective at improving group-level performance compared to traditional IPC; however, this improvement is likely owed to a decrease in non-response rather than an augmented ergogenic effect. Study 2 (Chapter 4) examined whether the beneficial effects of the early and late IPC windows can summate and further enhance the ergogenic stimulus of traditional IPC application. This experiment revealed that administering IPC in both windows is not capable of enhancing the ergogenic effect of traditional acute IPC.
The second focus of this thesis examined the role of perception modulation in the mechanism responsible for the ergogenic effect of IPC. Study 3 (Chapter 5) investigated whether IPC could reduce sensitivity to a cold-water pain, and if the degree of pain reduction would relate to the individual’s ergogenic response to IPC. Here, it was found that IPC did reduce cold-water pain sensitivity, however, these reductions were not related to IPC-mediated improvements in performance.

The last aim of this thesis evaluated the use of IPC as a tool that can be applied practically within a high-performance athletic training environment. Study 4 (Chapter 6) determined the impact of consistently, and repeatedly undergoing use of IPC as part of athletic training for improving endurance performance over time. It was revealed that highly trained athletes undergoing consistent and repeated IPC treatment within their training environment did not improve endurance performance over time.

Collectively, this thesis provides novel understanding into the optimal IPC methodology for maximizing the ergogenic effect, mechanisms responsible for the IPC effect, and the feasibility of IPC within a competitive environment. These insights are important for optimizing IPC as an ergogenic strategy.
7.2 Insights from an enhanced IPC stimulus

7.2.1 Enhancing the IPC stimulus does not augment the ergogenic effect.

Previous studies investigating new variations of the IPC protocol in an attempt to enhance the stimulus and augment the ergogenic effect have increased the number of cycles (92), length of cycles (93), and volume of muscle mass subjected to the stimulus (92). However, no variation was effective in augmenting the ergogenic effect beyond that of traditional IPC. In this thesis, I also attempted to enhance the IPC stimulus and augment the ergogenic effect by amplifying the metabolic stimulus (chapter 3) and by combining the beneficial effects of the early and late IPC windows (chapter 4).

In Chapter 3, it was demonstrated that on a group level, an amplified metabolic stimulus (i.e. IPC combined with either active walking or passive EMS) was effective at improving exercise performance compared to traditional IPC. However, traditional IPC itself did not improve exercise performance compared to control. In addition, examination of individual data revealed that of those who improved performance following traditional IPC (n=4), amplifying the metabolic stimulus by combining IPC with EMS or walking resulted in an augmented effect in only one participant. In contrast, those who did not improve performance following IPC (n=8), amplifying the metabolic stimulus by combining IPC with either EMS or walking resulted in a performance improvement in 5 and 7 of those 8 original non-responders, respectively. As such, the group-level performance improvement of an amplified metabolic stimulus compared to
traditional IPC is likely owed to more positive responses compared to control rather than an augmented ergogenic effect. This is in agreement with Crisafulli et al (38), who reported that amplifying the metabolic stimulus of IPC by occluding blood flow following light cycling augments exercise performance, but not beyond that of traditional IPC. In Chapter 4, it was revealed that enhancing the IPC stimulus by combining the beneficial effects of the early and late IPC windows also does not augment the effect of traditional IPC. Therefore, this thesis is in support of previous research that attempting to enhance the IPC stimulus does not further augment the ergogenic effect.

7.2.2 Enhancing the metabolic stimulus decreases IPC non-response

In Chapter 3, it was demonstrated that in a group of participants (n=8) for whom a traditional IPC stimulus was not effective, the amplification of a metabolic stress through EMS while under occlusion led to a subsequent increase in endurance performance (n=5). Further amplification of a metabolic stress through walking while under occlusion led to performance improvements in an even greater number of these participants (n=7). It is acknowledged that a required exercise intensity is necessary for exercise training to improve fitness and increasing exercise intensity eliminates the non-response to exercise training (115). Therefore, it is possible that IPC requires a certain metabolic threshold to be met before an ergogenic action can be exerted. Animal work supports the existence of a certain metabolic threshold as previous studies have indicated that sufficient metabolite accumulation (e.g., adenosine, bradykinin, and
opioids) is necessary to initiate the cytoprotective effect associated with IPC (15,17,178). As postulated above, amplifying the metabolic stimulus of IPC does not increase the ergogenic effect in a graded manner; therefore, IPC may instead act through an “all or none” metabolic threshold effect. Animal work also supports the existence of an “all or none” IPC phenomenon as a greater IPC cycle length or number does not result in a graded protection of I-R injury (179).

By methodological design, IPC is implemented in such a way that all individuals are subjected to full arterial occlusion; thus, it is unexpected that only certain individuals would reach a metabolic threshold if this were driven solely by alterations in vascular transport. Differences in muscle metabolite receptor sensitivity have been suggested to be a possible explanation for the high inter-individual variability in the metaboreflex response to exercise (180,181). Indeed, there appears to be genetic variability in skeletal muscle metaboreceptors and these genetic differences have been associated with the inter-individual variability in the metaboreflex response (182). Therefore, it is reasonable to suggest that certain individuals require a greater metabolic stimulus from IPC due to between-subject differences in metaboreceptor sensitivity. Future work is needed to better establish the existence of a metabolic threshold and potential between-subject differences.
7.2.3 The existence of responders and non-responders

In Chapter 4, it was demonstrated that on a group-level, traditional IPC was ineffective for increasing exercise performance, but upon examination of individual participants, over half responded to IPC to an extent greater than repeated-control variability. This finding, along with the preceding chapters, supports the notion that the presence of IPC responders and non-responders causes large within-study variability, which likely confounds interpretation of mean group data (91). Therefore, future studies investigating the effects of IPC on exercise performance should be aware of the existence of responders and non-responders to IPC and cautioned against merely analyzing by group means.

7.2.4 Explaining the existence of responders and non-responders

To date, explanations for differences in response to IPC remain unclear. Previous studies have reported a reduced IPC-mediated performance effect in women (52,89,100). However, this thesis does not support an effect of sex on IPC responsiveness, as sex was not related to the performance responders identified in chapter 4, although, it should be noted that this analysis was confounded by a low female sample size. Instead, a higher VO₂max was approaching significance in a strong association with being a true performance responder to traditional IPC, and this effect was not owed to highly fit individuals being able to produce a more consistent exercise performance. This is in agreement with the review by Incognito et al. (91), who reported
that the highest time-trial responder rates (~81%) were in the highly trained population. It remains unknown if the specific physiological characteristics responsible for an augmented VO$_{2\text{max}}$ (i.e. IPC oxygen delivery or uptake) explain the responsiveness, or rather if a higher VO$_{2\text{max}}$ is simply related to greater training experience. Future research is needed to better establish the influence of fitness status and training experience on the responsiveness to IPC.

In the current thesis, it was proposed that a metabolic threshold is needed to be met for IPC to exert ergogenic action. It is possible that IPC does not provide an adequate metabolic stimulus to reach this threshold in certain individuals which may provide explanation for the existence of responders and non-responders. Given that differences in VO$_{2\text{max}}$ were found to be related to IPC responsiveness, it is possible that a higher VO$_{2\text{max}}$ influences the ability to meet a threshold with traditional IPC. However, upon further analysis of data presented in chapter 3, it was found that the baseline fitness status of the participants who required a greater metabolic stimulus to elicit an IPC performance response (n=6; VO$_{2\text{max}}$=49 ± 3 ml.kg$^{-1}$.min$^{-1}$) was not statistically different than those who improved performance following traditional IPC (n=6; VO$_{2\text{max}}$= 46 ± 4 ml.kg$^{-1}$.min$^{-1}$; p= 0.3). However, it should be recognized that this comparison has a low statistical power and high probability of a type II error. Nevertheless, the inability of traditional IPC to provide a sufficient metabolic stimulus in
certain individuals should continue to be considered as a possible explanation for the heterogeneity in IPC response.

7.3 Perception modulation for improving performance

A clear metabolic mechanism explaining the beneficial effects of IPC has yet to be established; however, it has been hypothesized that IPC may modulate the perception of fatigue and improve exercise performance. Specifically, it has been proposed that IPC may reduce the metabolic sensitivity of group III/IV afferents and attenuate motor drive inhibition (81). However, this proposed mechanism has been challenged by other observations that suggest group III/IV afferent sensitivity is not reduced following IPC (83,84). Alternatively, IPC may modulate the perception of pain during a high-intensity exercise via local or remote release of endogenous opioids (86,87). Chapter 5 sought to determine whether IPC could reduce pain sensitivity to cold-water immersion of the hand, and if the degree of pain reduction would relate to the individual’s ergogenic response to IPC. Interestingly, IPC did reduce cold-water pain sensitivity, however, these reductions were not related to IPC-mediated improvements in performance. These findings suggest that reductions in pain sensitivity do not explain an individual’s ergogenic response following IPC. However, it should be considered that the mechanisms of “internal” pain perception during exercise are extremely complex and may not link to mechanisms by which IPC modulates an individual’s perception to an external painful stimulus. It is possible that IPC-mediated reductions in pain sensitivity
would have been related to IPC-mediated improvements in performance if the utilized
pain stimulus was more comparable to pain experienced during high-intensity exercise.

Chapter 5 reported that IPC administration prior to a cold-water immersion test
decreased total time under pain and it was found that this was mainly driven by a
reduction in the time it took for pain to subside after removal from the painful stimulus.
Previous clinical research also demonstrates that IPC can reduce post-operative pain.
Therefore, it is possible that IPC-mediated reductions in pain sensitivity relate more to
the recovery from a painful stimulus, which could have implications for sports that
include intermittent rest and recovery. Indeed, IPC has demonstrated to improve
perceptual recovery between repeated maximal jumps and sprints (89), and can reduce
perceived muscle pain 24-72h following a bout of eccentrically-damaging exercise
(164).

7.4 The use of IPC as a practical training tool

Short term, repeated IPC administration (7 days) has been shown to increase
skeletal muscle oxidative capacity and enhance microvascular oxygenated blood flow
(161,168). These adaptations have further been shown to be maintained over the long
term (8 weeks) when IPC is consistently applied (162). Additionally, it is known that IPC
can improve immediate endurance performance (91) and recovery from muscle
damaging exercise (164). Therefore, the use of long-term IPC should permit for an
enhanced endurance performance over time through either long-term physiological
adaptation, and/or through greater training outputs during each session. However, it was not found in Chapter 6 that in a group of highly trained endurance athletes, undergoing 8 weeks of consistent and repeated IPC treatment did not improve endurance performance. It is possible that an IPC-mediated effect was compromised by factors intrinsic to the athlete’s finely-tuned competitive environment. Specifically, all training was implemented by the coach, and high-intensity training often involved group sessions; thus, greater training outputs (or harder sessions, if the “IPC treated” athletes were feeling good) may not have been achieved if training intensity was controlled by group workouts. Furthermore, athletes did not restrict caffeine throughout the 8 weeks, despite previous evidence that caffeine can abolish the cytoprotective effect of IPC (94). As it is certainly impractical to exclude these factors during competitive training, the application of IPC within a typical athletic team setting may not be a feasible ergogenic training strategy, but it is possible a different effect would occur for an athlete who consistently trains at his or her own pace. It is also certainly possible that the use of long-term IPC simply does not cause physiological adaptation to enhance endurance performance over time and a strictly controlled study is needed to better establish this possible effect. Nevertheless, this finding questions the ecological validity of IPC use within an athletic setting and should encourage researchers to consider the practical applicability of IPC in their future investigations.
7.5 Limitations

The studies in the current thesis compared IPC interventions to a no intervention control and did not include a comparison to a placebo control group. Since the placebo effect can influence exercise performance (183), the data in the current thesis could have been influenced if participants believed the treatment would help. In all studies, it was ensured that all participants were naïve to the expected IPC outcomes; thus, it is likely that placebo effects were no more likely to occur than nocebo effect. In fact, despite this data not being included in this thesis, our group recently demonstrated that in a group of uninformed participants, the majority believed that IPC would negatively impact their performance compared to control, and interestingly, despite this negative expectation, IPC still improved performance (184). This finding suggests that placebo effects may be limited and that the performance benefits of IPC occur independently of performance expectations.

Previous studies have compared IPC to a sham intervention wherein cuffs are inflated to a low pressure (cuff pressure ranging from 10-50 mmHg) (37–39,64,82). However, such approach is limited as IPC induces pain during occlusion and relief during reperfusion, which does not occur during the low-pressure sham; thus, subjects are able to easily differentiate between conditions, possibly allowing for positive expectations to remain with IPC. In addition, it is possible that a low-pressure IPC can still elicit an ergogenic effect (43). The studies in the current thesis required many
separate trials (up to 7 trials) of difficult exercise tasks. Therefore, in order to avoid participant drop-out or motivation changes over multiple lab visits, a potentially ineffective sham control group was not included in the studies of this thesis. Nevertheless, the lack of a placebo group, which would help to confirm our findings, must be an acknowledged limitation to the current thesis. Future trials should attempt to develop an effective sham-control to minimize the possibility of placebo effects.

_A priori_ power calculations were not included in Chapters 3, 4 & 6 as the required variables (effect size, variability) that correspond to the exploratory variations of IPC administration were unclear. Therefore, the participant recruitment strategy in this thesis was based on the largest _n_ I could recruit given the constraints (budget, time, athlete availability), and _a-posteriori_ power analyses were performed. Low observed power was observed in certain statistical analyses; thus, if the observed effect sizes did indeed reflect the true effect sizes, there was insufficient power in these analyses to produce significant results. However, recruiting a sufficient number of participants to statistically detect these small effect sizes amongst large variation was an unrealistic goal, and perhaps an irrelevant goal given the observed magnitude of change was below the level that would be considered meaningful in the context of the athlete or the testing equipment. Nevertheless, low statistical power is an acknowledged limitation to certain parts of this thesis.
7.6 Future Directions

This thesis suggests that the inability of traditional IPC to provide a sufficient metabolic stimulus is leading to the presence of responders and non-responders, and that an amplified stimulus may be needed to ensure a metabolic threshold is met for IPC to exert ergogenic action. To test this hypothesis, a future study should investigate whether further increasing the metabolic stimulus of IPC (e.g. IPC combined with a higher walking intensity) can completely eliminate performance non-response. The findings of such experiment would provide valuable information to the coach, athlete, and researcher. For the coach and athlete, it would reveal whether everyone is able to respond to IPC, and if so, provide a method to ensure a positive response. For the researcher it would, depending on the outcome, direct future lines of research towards understanding between-subject differences in the metabolic threshold (e.g. differences in metaboreceptor sensitivity), or instead, toward investigating other factors (e.g. VO$_2$max, training experience) that could explain heterogeneity in the IPC response.

The current thesis does not support a role of pain sensitivity modulation in the mechanism responsible for the ergogenic effects, however, further research is needed to confirm this conclusion. Specifically, a future study should investigate whether IPC can reduce sensitivity to a painful stimulus that better mimics the pain experienced during a high intensity exercise bout (e.g. 5-minute isometric handgrip exercise @ 30% 1RM), and if the reduction of this type of pain would relate to individuals ergogenic
response. Current literature suggests that IPC may play a specific role in the recovery of a painful stimulus. Therefore, a future study should investigate whether IPC can improve repeated sprint performance by improving perceptual recovery (as measured by RPE) between bouts. This could have implications for the type of sport that would be best suited for the benefits of IPC.

7.7 Conclusion

This thesis supports the current literature that an enhanced IPC stimulus does not augment the ergogenic effect beyond the traditional protocol of three, 5-minute cycles of limb ischemia, followed by 5 minutes of reperfusion. However, amplifying the metabolic stimulus of IPC decreases the rate of performance non-response by ensuring that a metabolic stimulus threshold is met for IPC to exert an effect. Traditional IPC may not provide an adequate metabolic stimulus to reach the required threshold in every individual and this may provide explanation for the presence of responders and non-responders previously reported in the literature and confirmed in the current thesis. Although perception modulation remains a possible explanation for the beneficial effects of IPC, the current thesis does not support the role of IPC-mediated reduction in pain sensitivity as the mechanism responsible. Finally, the application of IPC within a typical athletic team setting may not be a feasible ergogenic training strategy. Overall, these findings provide novel insights into the optimization of IPC as an ergogenic strategy and important implications for future research.
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