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Bilateral Upper Limb Remote Ischemic Preconditioning

Improves Peak Anaerobic Power

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Bilateral Upper Limb Remote Ischemic Preconditioning Improves Peak Anaerobic Power

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Abstract

Bilateral Upper Limb Remote Ischemic Preconditioning

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Purpose: Ischemic preconditioning (IPC) has been used to protect myocardial cells against ischemia-reperfusion injury and is recently used for improving exercise performance. It is unknown whether a remote bout of IPC (RIPC) to tissue not involved in exercise can induce similar exercise improvements and what "dose" of IPC is necessary to induce exercise performance benefits. This study determined if unilateral and bilateral upper limb RIPC improves lower body anaerobic power output. **Methods:** Using two randomized, single blind, crossover study designs, we studied 43 young recreationally active adults. For study 1, unilateral RIPC was used and a sham control condition involved the inflation of blood pressure cuffs to 10 mm Hg. For study 2, the ischemic stimuli were increased to bilateral occlusion while the sham control condition used was 0 mm Hg of occlusion pressure. After the RIPC treatment, subjects completed four 30 s Wingate anaerobic tests on a Monark cycle ergometer with 2 min passive rest

between trials. **Results:** In the unilateral occlusion trial, peak power, mean power, and fatigue index were not different between the two conditions at every Wingate test. In the bilateral occlusion trial, peak power was elevated in the RIPC condition than in the sham control for the fourth Wingate test ($p<0.05$). Additionally, compared with the sham control, mean power was greater in the RIPC condition during the first and fourth Wingate tests (both $p<0.05$). **Conclusion:** Remote ischemic preconditioning applied bilaterally increased lower body power output over a series of Wingate anaerobic tests. Unilateral RIPC, however, had no effect on any of the performance variables, suggesting that there is a threshold for the amount of target tissue needed to elicit anaerobic performance benefits.

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CHAPTER 1

INTRODUCTION

1.1 Background

The extreme competitiveness and small margins that separate winners from losers have driven athletes to seek any substance, technique, or means that provide them with a competitive edge (60). In recent years, the utilization of so-called ergogenic aids is widely spread not only in elite athletes but also among regular exercisers. Indeed the biggest users of nutritional supplements, as well as anabolic steroids, are “gym rats” or recreationally active adults (60). Most of the frequently used, athletic performance enhancing ergogenic aids originate from clinical or medical use targeted at patient populations to ameliorate symptoms and conditions. One of the newest applications of a clinical utility applied for the purpose of aiding performance is ischemic preconditioning (IPC) (17,32). Ischemic preconditioning is performed by applying alternating bouts of ischemia and reperfusion, typically to myocardial tissue, and has been shown to delay cardiac cell injury following a subsequent ischemic insult (46). The development of remote ischemic preconditioning (RIPC) has since provided a noninvasive, clinically applicable method for preconditioning of ischemic myocardium through remote occlusion of the artery (53,36,7).

IPC is very attractive as an ergogenic aid in several aspects as it is non-invasive, legal, easy to apply, and avoids deleterious side effects of other ergogenic aids. In one of the original studies to address this, bilateral lower extremity IPC improved maximal

oxygen consumption by 3% in well-trained cyclists (17). However, the effects of IPC on maximal oxygen consumption have not been replicated in subsequent studies (16,2,32). A more promising application of IPC appears to be in exercise events that involve anaerobic power output as IPC has been demonstrated to increase resistance to hypoxic injury and ischemic tolerance (55). In incremental maximal cycling tests, maximal oxygen consumption did not change but maximal workload and total exercise time increased with the IPC application (16), suggesting that IPC might have increased anaerobic capacity. Bilateral upper extremity IPC elicited an improvement in 100 m swim time in Olympic level swimmers (32). To date, previous studies involving IPC and exercise performance have all used localized IPC, applying IPC to the tissues subsequently used for exercise performance. To our knowledge, no study has examined whether a remote bout of IPC to tissue not involved in exercise (i.e. upper extremity IPC prior to lower body exercise) is capable of improving exercise performance. Moreover, it is not clear what "dose" of RIPC is necessary to induce exercise performance benefits.

Accordingly, we tested the hypothesis that RIPC of the upper extremity would confer systemic benefits and produce improvements in lower body anaerobic power output. Because many sporting events are performed in an intermittent fashion, we implemented 4 bouts of anaerobic tasks in a row, to see if the effects of RIPC could persist through multiple bouts of exercise.

1.2 Statement of Purpose

The purpose of the present investigation was to determine if remote ischemic preconditioning of the upper extremity would induce improvements in lower body exercise performance over the course of four repeated Wingate anaerobic power tests. The specific objectives of the study were to:

1. Determine whether upper extremity remote ischemic preconditioning, applied either bilaterally or unilaterally, would improve peak or mean power as assessed by repeated Wingate anaerobic tests.

2. Determine whether there exists a "threshold" amount of tissue that must be subjected to remote ischemic preconditioning to confer exercise benefits.

1.3 Hypothesis

In the current study, we tested the following hypotheses:

1. Bilateral remote ischemic preconditioning of the upper extremity would improve lower body power output versus a sham control.

2. Unilateral remote ischemic preconditioning of the upper extremity would also improve lower body power output versus a sham control.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Beginnings of Ischemic Preconditioning

Murry, Jennings, and Reimer first reported the role of ischemic preconditioning as a means of providing cardio protective benefits. By using an IPC protocol consisting of alternating bouts of occlusion and reperfusion of the circumflex artery, they discovered that infarct size following a sustained bout of ischemia was reduced by 25% versus a control group of canines (19). Przyklenk et al. were the first to show that "remote" occlusion of one vascular bed (circumflex artery) could reduce the infarct size in "virgin" myocardium observed after sustained occlusion of the left anterior descending artery, thus establishing remote ischemic preconditioning (RIPC) (22). Since that time, remote bouts of IPC have been shown to offer protection against ischemia/reperfusion injury by reducing infarct size through occlusion of various non-cardiac vascular beds, including renal artery occlusion (42) and anterior mesenteric artery occlusion (24). However, due to the invasive and dangerous nature of applying preconditioning to such vascular beds, the implications of these protocols were perhaps limited outside of a clinical setting.

The first instance in which RIPC was applied to skeletal muscle was in an experiment done by Birnbaum, Hale and Kloner in 1997. They established RIPC by combining a restriction of femoral arterial blood flow via stenosis and electrical stimulation of the gastrocnemius. Through this process of RIPC, myocardial infarct size

expressed as a fraction of the ischemic risk zone was reduced by 65% following a sustained bout of coronary artery occlusion and reperfusion (5). Furthermore, Oxman et al. were the first to induce RIPC in a noninvasive protocol by applying a tourniquet to the hind limb of rats, after which they reported a reduction in reperfusion arrhythmias following an ischemic event (48). Kharbanda et al. continued to establish a more practical form of inducing RIPC by using a blood pressure cuff for ischemia/reperfusion purposes, preventing ischemia-reperfusion induced endothelial dysfunction in humans and reducing myocardial infarct size in pigs (17). Since that time, the common protocol for inducing cardio protection remotely has involved using 5-minute alternating cycles of inflation (occlusion) and deflation (reperfusion) of blood pressure cuffs applied to skeletal muscle at around 220 mmHg.

2.2 Physiological Mechanisms Underlying IPC, Clinically

Although the exact mechanism through which IPC confers protection against potential ischemia-reperfusion injury is not fully elucidated, there are three main hypotheses that likely work in an synergistic fashion in order to confer these cardio protective benefits, namely a systemic response, a neural pathway, and a humoral pathway. The systemic response is thought to occur two-fold through inflammatory pathways, by suppressing the inflammatory response itself (51) and favoring a gene transcription profile that is both anti-inflammatory and anti-apoptotic (37).

Gho et al. established the first involvement of a potential neural pathway, administering a known ganglion blocker, hexamethonium, to abolish the reductions in myocardial infarct size seen following IPC (24). Since that time, a select number of endogenous substances, such as adenosine, have been implicated in the neural pathway. These substances are thought to convey cardio protection by activating local muscle afferent pathways within the remote preconditioned organ to then stimulate efferents terminating at the heart (28). Administration of adenosine receptor antagonists have been found to abolish the protective effects of IPC on myocardial infarct size (30,50), but on the other hand administration of adenosine itself has been shown to confer cardio protection, enhance protective effects, and maintain ATP levels following an ischemic event (40,11,30). Lastly, an intact neural pathway was required for inducing IPC cardio protection in a rat model, further providing evidence for IPC to be modulated by a neural pathway (20).

Evidence for a humoral factor was first demonstrated through the transfer of blood from a preconditioned rabbit to an untreated rabbit, reducing myocardial infarct size by 77% (19). The "washout" of substances into the bloodstream during the reperfusion period of preconditioning is thought to confer cardio protective benefits, allowing the substances to bind specific receptors and initiate the preconditioning effect, as opposed to stimulating local afferents (28). Although the actual identity of the humoral mediator is at this point unclear, numerous endogenous substances such as adenosine (50) have been implicated.

2.3 Current Role of IPC in Exercise Performance

IPC has become very attractive as an ergogenic aid in several aspects due to the non-invasive, legal, and easy to apply protocol, while it also avoids deleterious side effects of other ergogenic aids. Bilateral lower extremity IPC was first used in an exercise setting by de Groot et al., who applied an incremental maximal cycling test following either IPC or a sham control in healthy- well-trained cyclists. The IPC protocol improved maximal oxygen consumption (VO_{2max}) by 3% and elicited a 1.6% improvement in power output over the course of the test (17). In another study using incremental maximal cycling tests, maximal oxygen consumption did not change but maximal workload and total exercise time increased with the IPC application (16). Furthermore, in a study incorporating multiple exercise bouts, IPC elicited a 34 s improvement in 5 km time trial effort. 45 minutes preceding the 5 km time trial, subjects also undertook a maximal running test, in which no improvements were found in the IPC group compared to a sham control (2).

Since the effects of IPC on maximal oxygen consumption have not been replicated in subsequent studies (16,2,32), the combination of these studies suggests that IPC might have increased anaerobic capacity during these protocols. Hence a more promising application of IPC appears to be in exercise events that involve anaerobic power output. IPC has been demonstrated to increase resistance to hypoxic injury and ischemic tolerance (55). Thus far however, research of a solely anaerobic performance following

IPC is limited, only producing a mean improvement of 0.7 seconds in maximal swim time over 100 m (32).

2.4 Wingate Anaerobic Threshold Test

The Wingate test is considered a valid and reproducible method of assessing anaerobic power, and thus is commonly used in the field of exercise physiology (4). The test consists of an all-out 30-second sprint on a cycle ergometer against a frictional resistance relative to the individual's body weight, commonly between 7.5 and 9% (5). Although variations of the Wingate test are numerous, typically either 10 s or 30 s Wingate tests are most common. One of the primary outcome measures of the Wingate test is peak power. Peak power is normally elicited in the first 3-5 seconds of the test and demands a large output of ATP, predominantly supplied through phosphocreatine (PC) stores (4). Therefore, in order assess the connection between the ATP-PC energy system, IPC, and peak power performance, a 10 s Wingate may be the most relevant testing mechanism. However, higher post-exercise heart rate and higher lactate acid concentrations have been noted following a 30 s Wingate, demonstrating that this particular test may be the most beneficial method of exploring total anaerobic capacity in relation to IPC (63). Energy from the metabolism of anaerobically produced lactic acids during Wingate tests has been shown to explain 81-83% of the variances for peak and mean power output, thereby providing further evidence for the use of Wingate to assess anaerobic capacity (6).

Another important component of the Wingate test is the ability to use it in an intermittent nature. A variety of widely popular team sports, such as soccer, are characterized by a number of short sprints followed by low-to-moderate levels of activity or passive rest (43). The ability of athletes to perform short-duration sprints interspersed with short recovery periods is therefore particularly relevant in most athletic situations. To our knowledge, there is no existing data regarding IPC's effects on an intermittent style of exercise. Therefore, having subjects undertake four consecutive 30 s Wingate bouts, separated by two minutes of passive recovery, would provide some insight into the role of IPC on intermittent power output.

2.5 Physiological Mechanisms Underlying IPC's Effects on Exercise Performance

What are the physiological mechanisms underlying the effects of IPC on anaerobic performance? Similar to the likely synergy of multiple pathways helping to elicit cardio protection in a clinical setting, a number of different factors may contribute to improving exercise performance. High-level exercise performance is known to be limited by skeletal, cardiac and respiratory muscle fatigue associated with episodes of exercise-induced arterial hypoxemia, analogous to a physiologic form of ischemic injury. However, much speculation in the current literature tends to implicate the involvement of ATP sensitive potassium channels (K_{ATP}), as administration of K_{ATP} channel antagonists have been found to diminish preconditioning effects, while administration of K_{ATP} channel agonists increase preconditioning effects (30). Therefore any modulators that

center on K_{ATP} channels may have a profound effect in explaining IPC's benefit on exercise improvement.

A number of modulators at the endothelial level may help to mediate the preconditioning pathway. Adenosine has been found to be rapidly produced by the endothelium during ischemia (45). While adenosine is widely known to cause vasodilation, the presence of adenosine is also responsible for mediating a cascade of effects to further enhance skeletal muscle vasodilation. As described previously, adenosine is responsible for opening K_{ATP} during exercise through the activation of protein kinase C. The opening of K_{ATP} channels initiates functional sympatholysis (33), a mechanism of the utmost importance during exercise to reduce blood flow to splanchnic tissue and re-direct blood flow to actively used skeletal muscle.

Ischemic preconditioning also has been shown to improve nitric oxide (NO) availability in healthy adults (35). Similar to adenosine, NO is a known factor for endothelium-dependent vasodilation, providing a potential stimulus to increase in perfusion to working skeletal muscle during exercise (38,62), as well as reduce mitochondrial oxygen consumption through the use of an oxygen extraction reserve (52). The creation of an oxygen reserve at the mitochondrial level could potentially increase its bioavailability for later use, helping to maintain a higher arterial-venous difference in order to improve VO_{2max}. Once again similar to adenosine, NO is thought to trigger the opening of K_{ATP} channels, modulated through protein kinase C activation, in order to stimulate the same protective effects after preconditioning (51).

To maintain the high level of power needed to accomplish a number of repeated Wingate sprints, PC stores must be replenished, which is typically only thought to happen during periods of recovery and is dependent on the availability of oxygen and aerobic metabolism (9). Studies have shown that power decrement occurs at a much greater rate in athletes with lower VO_{2max} (8), establishing a connection between PC resynthesis, $VO_{2\ max}$ and recovery of power output. While studies have shown have shown that most young adult men can recover fully from a Wingate test within 10 minutes after completion (29), tests incorporating repeated-sprint ability in cyclists revealed PC utilization to increase from 50% to 80% between sprint one and sprint ten, whereas total PC concentration decreased from 57% of its initial value after sprint one to 16% after the final sprint (57). This suggests that in shorter periods of rest, PC resynthesis does not occur fast enough to fully replenish stores and that a reduction in power should be expected to follow. Creating an oxygen reserve through NO pathways may help to preserve PC for further use during repeated Wingate efforts.

The influence of the phosphagen system (ATP and PC) may play a larger role in the improvement in power output than is perhaps explored in the current literature, as phosphagen contents in the skeletal muscle may have been elevated by the RIPC stimuli. In a few initial clinical studies of IPC, higher muscle contents of ATP and PC, and a lower muscle content of lactate were reported following preconditioning (61,49,46). Furthermore, these initial studies concluded that preconditioning induced a reduction in energy demand in the myocardium following a prolonged bout of ischemia (47). More

specifically, although preconditioned myocardium started with a reduced level of ATP, preconditioned myocardium exhibited a slower rate of ATP utilization, high energy phosphate utilization, glycogenolysis, and anaerobic glycolysis (47). Higher muscle contents of PC, ATP, and a lower muscle content of lactate following preconditioning have been replicated in further studies (49).

During hypoxic conditions during exercise, anaerobic metabolism is the system predominantly contributing to energy production. As mentioned previously, energy from the metabolism of anaerobically produced lactic acids during Wingate tests has been shown to explain 81-83% of the variances for peak and mean power output (6). Lactate accumulation may be delayed through the uncoupling of oxidative phosphorylation by opening mitochondrial K_{ATP} channels (23). One proposed explanation of improved may be that IPC not only lowers muscle content of lactate following preconditioning, but IPC could contribute to an increased removal of lactic acid (15,35) or an up-regulation of both intra- and extracellular lactate shuttles during exercise (10,27,54). However, although IPC has been shown to delay lactate accumulation during a submaximal incremental running test (2), there have been no changes reported in blood lactate concentrations in other exercise performance protocols following IPC (14,16,17,32), seemingly suggesting that blood lactate concentrations do not appear to be modulated by IPC.

The preservation of ATP and reduction in high-energy phosphate utilization could also contribute to the attenuation of muscle fatigue, and thus provide an explanation for the increase in power output seen in previous studies, through the modulation of K_{ATP}

channels. K_{ATP} channels have four inwardly rectifying potassium channel subunits (Kir6.x, in two isoforms Kir6.1 and Kir6.2) forming a central pore surrounded by four regulatory sulphonylurea receptor subunits (SUR) (13,44). ATP is known to close K_{ATP} channels by binding to Kir6.2 (5). In previous studies, a blockade of K_{ATP} channels via glibenclamide both attenuated the decline of tetanic force and $[Ca^{2+}]_I$ in fatigued muscle (21,26) and demonstrated a myoprotective effect in protecting skeletal muscle (26). Furthermore, other studies have shown that either a blockade of K_{ATP} channels through glibenclamide administration or a knockout of Kir6.2 genes to produce K_{ATP} channel deficient mice were sufficient in reducing the increase in resting tension of the muscle during fatigue (25,41). Although it is widely known that K_{ATP} channels are activated during ischemia, hypoxia, and metabolic inhibition, it is likely that the increase and preservation of ATP produced by preconditioning may contribute to an attenuation of the opening of K_{ATP} channels to an extent so as to reduce muscle fatigue during a series of Wingate tests.

2.6 Relevance and Novelty of RIPC on Maximal Exercise Performance

The pressure to constantly improve exercise performance in the arena of competitive athletics is well known. As athletes are more frequently being paid more money on the basis of their performance, it is no surprise that most are willing to exhaust any possibility to enhance performance and achieve gaining a small advantage over a competitor that could make the difference between a gold medal and a silver medal. However, in recent years, the utilization of ergogenic aids is widely spread, not only in

elite athletes, but also among regular exercisers. Indeed, the biggest users of nutritional supplements, as well as anabolic steroids, are "gym rats" or recreationally active adults (60). Although most of the frequently used, athletic performance enhancing ergogenic aids originate from clinical or medical use targeted at patient populations to ameliorate symptoms and conditions, it is not uncommon for athletes to utilize methods like living at high altitudes and training at low altitudes in order to enhance the oxygen content of their blood. Nevertheless, most strategies involve some sort of ramification, some as little as changing places of residence just for training purposes to methods that result in deleterious side effects and health hazards. Hence, IPC may offer a "natural doping" mechanism that athletes may find appealing.

Many of strategies, such as training at altitude produce improvements on the scale of 2-4% (39,58). Thus far, the improvements induced by IPC on exercise performance are on a similar scale (17,2). From a biostatistical standpoint, a 2-4% increase in athletic performance may seem minimal, but sports are often decided by the smallest of margins. To provided examples, Usain Bolt won the 100 m sprint in the 2008 Beijing Olympic games in a dominating fashion while setting a new world record, but the difference between the gold and silver medal times was only 2%. In those same Olympics, Michael Phelps won the 100 m butterfly by 0.01 s, but the difference between the gold medal and 8th place was 2.5%. Thus, from the athletic performance point of view, the improvements of 2-3% may be substantial.

To this date, studies investigating the role of IPC in exercise performance have applied IPC bilaterally and locally to the same musculature that is subsequently used in exercise (i.e. applying IPC to the lower body before having subjects complete a 5-km time trial on a treadmill) (16,17,2,32). Remote IPC has been well documented to confer a variety of positive systemic effects in a clinical setting (55). For example, IPC applied to the legs can prevent a decrease in brachial artery endothelial function, indicating a systemic, rather than localized, effect of RIPC (1). However, to our knowledge, no study has examined whether a remote bout of IPC to tissue not involved in exercise (i.e. upper extremity IPC prior to lower body exercise) is capable of improving exercise performance.

At the same time, previous studies in the field have applied bilateral occlusions at a substantially high pressure (220 mm Hg) to establish arterial occlusion (16,2,14), but a higher occlusive pressure of 250 mm Hg has been speculated to not fully block arterial flow to the lower extremity (31). Additionally, anecdotal evidence indicates that leg occlusion elicits much greater pain than arm occlusion. Due to the smaller amount of tissue in the upper extremity, far less pressure is needed for full arterial occlusion.

Moreover, it is not clear what "dose" of RIPC is necessary to induce exercise performance benefits. Clinical studies have demonstrated a potential benefit of unilateral IPC, as remote preconditioning of one limb prevented subsequent ischemia-reperfusion (IR) induced endothelial dysfunction in the contralateral limb (34), and ischemia-reperfusion to one arm produced vasodilatory affects in the brachial artery of the

contralateral arm (22). These results indicate that a greater amount of IPC would be required to elicit sufficient systemic effects to produce necessary benefit as an ergogenic aid.

The application of either a single or double blood pressure cuff to the upper extremity could provide a more practical, less painful, and widely applicable protocol, should the strategy confer similar improvements of exercise performance as locally-applied IPC. Bilateral upper extremity occlusion could be easily implemented before competitions at a relatively low cost and amount of effort on the user part. Furthermore, if RIPC demonstrates improvements of power over a series of anaerobic challenges, the potential for widespread use in sports such as soccer and basketball could provide a unique entrepreneurial opportunity for businesses, or also in future clinical settings to aid exercise in populations such as cardiovascular disease patients, disabled patients, elderly patients, or others that might be restricted from exercise.

CHAPTER 3

METHODOLOGY

3.1 Study Design

In order to evaluate the effects of RIPC on anaerobic exercise performance as comprehensively as possible, we conducted 2 different but complimentary studies. Within each study, we used a controlled, randomized, single-blinded, crossover experimental design with two experimental conditions. For study 1, unilateral remote ischemic preconditioning was used and a sham control condition involved the inflation of blood pressure cuffs to 10 mm Hg. For study 2, the ischemic stimuli were increased to bilateral occlusions. Additionally, to eliminate a possibility of the low blood pressure cuff inflation inducing any ischemic conditioning effects in the sham control condition, the cuff was placed but was not inflated during the sham control for study 2. Other experimental procedures were identical between the 2 studies.

3.2 Subjects

A total of 43 young, healthy, recreationally active subjects were studied in the present study. For study 1, 14 healthy, recreationally active adults (6 males, 8 females) participated. For study 2, 29 healthy adults (21 males, 8 females) volunteered to participate. Selected subject characteristics are displayed in Table 1. Exclusion criteria employed for both studies were: participation in greater than 420 min of vigorous exercise per week, medication usage, regular smoking, and chronic disease as assessed by a Health Research Questionnaire. The Institutional Review Board at the University of

Texas at Austin reviewed and approved the study. All volunteers gave their written informed consent before participation.

3.3 Experimental Protocol

To ensure that subjects could reliably complete the exercise protocol, all subjects performed two familiarization trials, completing two 30 s Wingate tests (59) separated by two minutes of passive recovery in the first familiarization, and four 30 s Wingate tests separated by two minutes of passive recovery in the second familiarization. The main experimental protocol was performed at least a week after the familiarization sessions. All study activities were performed under ambient temperature (22.4°C), humidity (37%), and pressure (757 mm Hg) in a controlled laboratory setting. For each testing visit, subjects reported to The University of Texas Cardiovascular Aging Research Laboratory at the same time of day each time to eliminate any potential diurnal effects on power output (56). Additionally, subjects were fasted for at least four hours and had abstained from alcohol, caffeine, and vigorous physical activity for at least 24 h before testing. Upon arrival, participants sat quietly for 5 min in a controlled environment and baseline-resting blood pressure was measured (Omron HEM-907, Netherlands). The test protocol employed in the present study is displayed in Figure 1. While in the supine position, an automated inflatable cuff (E20 Rapid Cuff Inflator, D.E. Hokanson, Bellevue, WA) was positioned unilaterally in study 1, on the left upper arm, and bilaterally in study 2, on the left and right upper arms, after which participants received four, 5 min bouts of RIPC or sham treatment, followed each by 5 min of reperfusion. On the subsequent visit, participants completed identical procedures except undergoing the

alternative condition. Although participants were likely aware of pressure differences between conditions, they remained naïve to the rationale of the experiment. To ensure that the occlusion and reperfusion of tissue were properly achieved, finger temperature was monitored throughout both conditions (DTM Raw Data Acquisition, Endothelix, Houston, TX) (18).

Prior to the exercise protocol, subjects engaged in a 5 min warm-up. During this warm-up period, subjects began pedaling comfortably against no resistance at 60-75 RPM (~50 W) on a cycle ergometer (Monark Ergomedic 894E Peak Bike, Netherlands) and executed an intermittent sprint achieving maximal voluntary RPM at minutes 2, 3, and 4, respectively. Following the warm up, participants rested quietly for 5 min before performing the Wingate exercise (59).

Fifteen minutes after each experimental condition, participants completed four Wingate anaerobic tests on a specialized cycle ergometer (Monark Ergomedic 894E Peak Bike, Netherlands). Each test lasted 30 s in duration, followed by 2 min of passive rest. Subjects cycled from rest to 150 RPM, after which a frictional resistance of 9% body weight was instantly applied to the flywheel. Subjects received no verbal encouragement during each test. Five minutes following the final exercise bout, blood lactate was collected (LactatePro, Arkray, Kyoto, Japan). Peak power (W), mean power (W), and fatigue index (%)([(peak power – ending power)/peak power]*100) were calculated for each Wingate. Total (composite) power output (W)(mean power per Wingate * 30 s * 4 sets) and overall fatigue index (%) ([(peak power 1st Wingate – ending power 4th Wingate) / peak power 1st Wingate]*100) were also calculated.

3.4 Statistical Analyses

Differences in exercise performance variables between conditions were assessed with paired *t*-tests. All data were analyzed using SPSS statistical analysis software version 22.0 (SPSS Statistics, IBM, Armonk, NY). For all analyses, significance was set *apriori* at $p<0.05$. Data are presented as means ± SD.

CHAPTER 4

RESULTS

4.1 Results

As shown in Table 2, both peak and mean power outputs were greater in study 2 (bilateral RIPC) than in study 1 (unilateral RIPC), owing to the greater percentage of male subjects in study 2. In both studies, peak and mean power demonstrated substantial reductions from Wingate 1 to Wingate 4 where the fatigue index remained fairly constant. In study 1, peak power, mean power, and fatigue index were not different between the two conditions at every Wingate test. Similarly, total (composite) power output, overall fatigue index, and blood lactate concentration were not significantly different between the two conditions (Table 3).

In study 2, which utilized bilateral RIPC, peak power was significantly greater in the RIPC condition than in the sham control for the fourth Wingate test ($p<0.05$) (Figure 2). Additionally, compared with the sham control, mean power was significantly higher in the RIPC condition during the first and fourth Wingate tests (both $p<0.05$) (Figure 3). There were no significant differences between the conditions for total (composite) power output, overall fatigue index, or blood lactate concentrations (Table 3).

Table 1. Selected subject characteristics.

Characteristics	**Unilateral RIPC**	**Bilateral RIPC**
n (males, females)	14 (6,8)	29 (21,8)
Age (years)	22.2 ± 5.3	23.2 ± 3.8
Height (cm)	168 ± 7	175 ± 6
Body Mass (kg)	66.3 ± 10.7	72.2 ± 10.6
BMI (kg/m^2)	23.4 ± 2.5	23.5 ± 3.5
Systolic Blood Pressure (mm Hg)	118 ± 11	119 ± 11
Diastolic Blood Pressure (mm Hg)	67 ± 8	71 ± 6
Physical Activity (min/week)		
Low Activity	79 ± 75	87 ± 108
Moderate Activity	146 + 110	106 ± 85
Vigorous Activity	189 ± 93	219 ± 163
Total Activity	414 ± 192	413 ± 201

Values are expressed as means ± SD

RIPC = remote ischemic preconditioning; BMI = body mass index.

Table 2. Anaerobic power outputs as measured by Wingate anaerobic power tests.

Unilateral RIPC	**Wingate 1**	**Wingate 2**	**Wingate 3**	**Wingate 4**
Peak Power (W)				
Sham Control	591.7 ± 176.2	502.1 ± 144.2	473.9 ± 117.4	451.1 ± 121.4
RIPC	565.7 ± 174.3	513.2 ± 182.0	470.7 ± 133.6	448.0 ± 122.6
Mean Power (W)				
Sham Control	427.2 ± 96.2	366.0 ± 90.0	334.9 ± 72.6	318.9 ± 73.5
RIPC	409.5 ± 98.3	363.8 ± 92.6	330.0 ± 75.2	317.9 ± 68.2
Fatigue Index (%)				
Sham Control	50 ± 10	46 ± 11	49 ± 11	50 ± 12
RIPC	48 ± 11	47 ± 12	50 ± 12	50 ± 10
Bilateral RIPC	**Wingate 1**	**Wingate 2**	**Wingate 3**	**Wingate 4**
Peak Power (W)				
Sham Control	740.0 + 230.0	660.0 ± 183.3	584.0 ± 159.9	533.8 ± 128.3
RIPC	752.5 ± 213.6	664.0 ± 187.0	579.8 ± 148.3	551.0 ± 142.3*
Mean Power (W)				
Sham Control	527.4 ± 159.4	448.6 ± 114.5	397.0 ± 98.6	369.2 ± 76.2
RIPC	540.3 ± 160.9*	452.1 ± 116.9	396.0 ± 96.9	379.3 ± 88.4*
Fatigue Index (%)				
Sham Control	54 ± 10	55 ± 12	55 ± 13	54 ± 13
RIPC	54 ± 11	55 ± 11	56 ± 13	54 ± 15

Values are expressed as means ± SD. *$p<0.05$ vs. Sham Control. RIPC = remote ischemic preconditioning

Table 3. Composite measures of Wingate anaerobic power outputs and post-exercise blood lactate concentrations.

Unilateral RIPC	
Total Power Output (W)	
Sham Control	42,435 ± 8,650
RIPC	41,875 ± 9,015
Total Fatigue Index (%)	
Sham Control	60 ± 15
RIPC	59 ± 14
Blood Lactate (mmol/L)	
Sham Control	13.3 ± 3.2
RIPC	13.6 ± 2.7
Bilateral RIPC	
Total Power Output (W)	
Sham Control	52,381 ± 12,932
RIPC	53,081 ± 13,429
Total Fatigue Index (%)	
Sham Control	67 ± 11
RIPC	66 ± 12
Blood Lactate (mmol/L)	
Sham Control	13.4 ± 1.4
RIPC	13.0 ± 2.0

Values are expressed as means ± SD.

RIPC = remote ischemic preconditioning

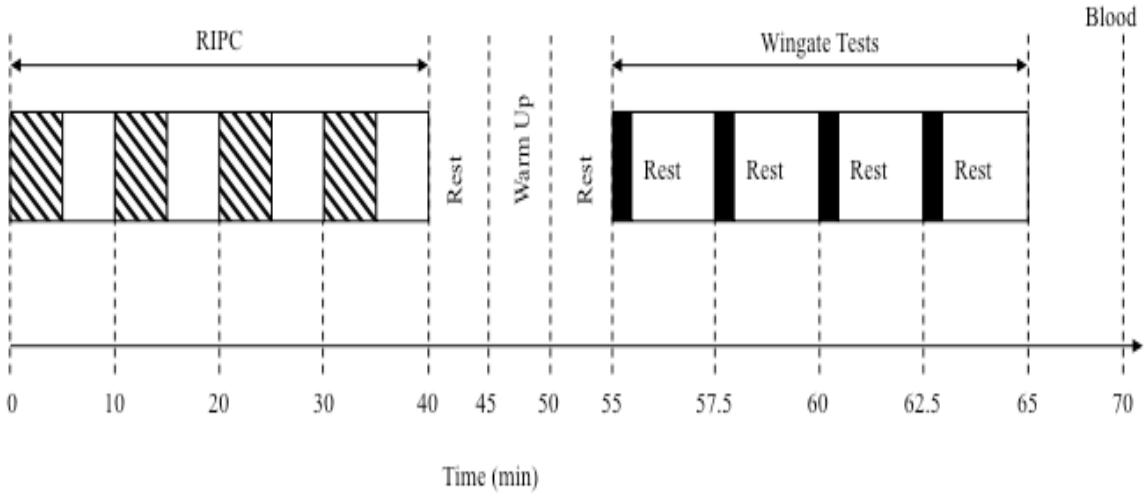

Figure 1 – RIPC test protocol. RIPC represents RIPC treatment in which striped boxes represent 5 min. of ischemia, white boxes represent 5 min. of reperfusion. Wingate tests represents Wingate performance in which black boxes represent 30 s of Wingate exercise, rest boxes represent 2 min. rest.

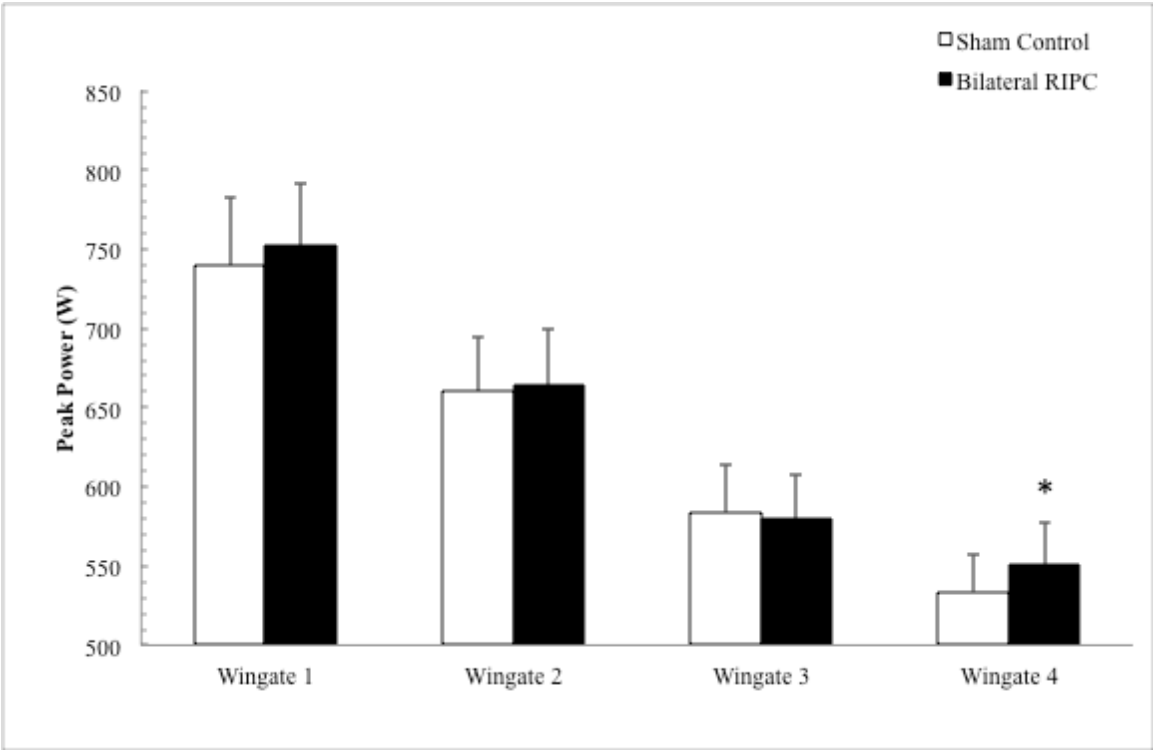

Figure 2 – Peak Wingate anaerobic power in the Sham Control vs. Bilateral RIPC groups. Data are presented as means ± SEM. *p<0.05 vs. Sham Control.

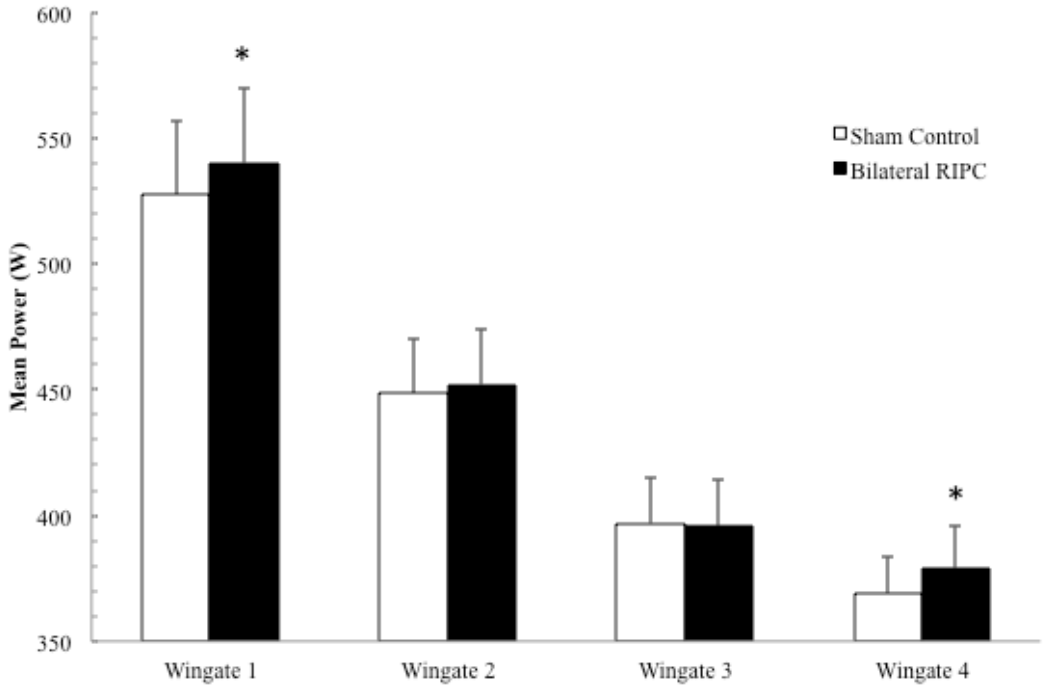

Figure 3 – Mean Wingate anaerobic power in the Sham Control vs. Bilateral RIPC groups. Data are presented as means ± SEM. *p<0.05 vs. Sham Control.

CHAPTER 5

DISCUSSION

The major finding of the present study is that bilateral remote ischemic preconditioning of the arms significantly increased lower body anaerobic exercise performance. The improvements were observed in both mean and peak power output, as assessed by the well-established Wingate anaerobic power tests. To our knowledge, this is the first study to demonstrate benefits of remote ischemic preconditioning stimuli on anaerobic exercise performance. In contrast, unilateral RIPC had no effect on any of the performance variables, suggesting that there may be a threshold for the amount of remote ischemic stimuli needed to elicit anaerobic performance benefits.

In a clinical setting, RIPC has been well documented to confer a variety of positive systemic effects (55). For example, IPC applied to the legs can prevent a decrease in brachial artery endothelial function, indicating a systemic, rather than localized, effect of RIPC (1). To this date, studies investigating the role of IPC in exercise performance have applied IPC locally to the same musculature that was subsequently used in exercise (e.g., applying IPC to the lower body before having subjects complete a running time trial) (16,17,2,32). Our data add novel evidence that remote IPC stimuli can confer significant benefits on exercise performance similar to locally applied IPC. Specifically, we observed an increase in mean power output in the first and fourth Wingate tests and an increase in peak power output in the fourth Wingate test, demonstrating that bilateral

upper extremity RIPC can improve lower-body power output over a series of highly anaerobic exercise challenges. Thus, the present results considered together with previous findings highlight the potential application and use of RIPC in the competitive athletic setting.

The previous studies in the field have required the use of a substantially high pressure (220 mm Hg) to establish arterial occlusion of the lower body musculature (17,2,14), but a higher occlusive pressure of 250 mm Hg may not fully and completely block arterial flow to the lower extremity (31). Due to the smaller amount of tissue in the upper extremity, far less pressure is needed for full arterial occlusion. Additionally, anecdotal evidence indicate that leg occlusion elicits much greater pain than arm occlusion. Thus, IPC of the upper extremity employed in the present study provides a more practical, less painful, and widely applicable protocol for a variety of athletes.

Clinical studies have demonstrated a potential benefit of unilateral IPC, as remote preconditioning of one limb prevented subsequent ischemia-reperfusion (IR) induced endothelial dysfunction in the contralateral limb (34), and ischemia-reperfusion to one arm produced vasodilatory affects in the brachial artery of the contralateral arm (22). We reasoned that unilateral IPC would be a more convenient and less painful way to apply RIPC and would produce the effects on anaerobic performance similar to those observed in previous clinical studies (34). However, unilateral IPC applied remotely had no significant effects on anaerobic performance in the present study. These results indicate that a greater amount of IPC would be required to elicit sufficient systemic

effects to produce necessary benefit as an ergogenic aid. It should, however, be noted that there are several other differences between the unilateral and bilateral studies. For example, in the unilateral IPC trial, we used an occlusion pressure of 10 mmHg in the sham control condition. It is possible that the application of a low-pressure cuff may have caused a similar effect of IPC to a greater occlusion pressure as speculated in a previous study (16).

The magnitude of improvements in anaerobic exercise performance we observed in the present study (2-3%) was seemingly very small but is in line with the 2-3% increases reported by previous studies that have addressed the effects of ischemic preconditioning on aerobic performance (17,2). Viewing from a biostatistical standpoint, such small increases seem negligible, but athletic competition is often decided by a very small margin of differences. Usain Bolt won the 100 m sprint in the 2008 Beijing Olympic games in a dominating fashion while setting a new world record, but the difference between the gold and silver medal times was only 2%. Thus, from the athletic performance point of view, the improvements of 2-3% may be substantial.

What are the physiological mechanisms underlying the effects of RIPC on anaerobic performance? During hypoxic conditions as well as in strenuous exercise, anaerobic energy system, in particular glycolysis, contributes predominantly to energy production during strenuous exercise. In the Wingate anaerobic tests, energy from the metabolism of anaerobically produced lactic acids has been shown to explain 81-83% of the variances for peak and mean power output (6). The uncoupling of oxidative

phosphorylation by opening mitochondrial K_{ATP} channels has been suggested to be a physiological mechanism underlying the effect of IPC on reducing oxidative damage and is known to reduce and delay lactate accumulation (13). However, blood lactate concentrations do not appear to be modulated by IPC (16,17,32,14). Indeed blood lactate concentration was not different between the RIPC and sham control conditions in the present study.

An alternative explanation is that phosphagen (ATP and CP) contents in the skeletal muscle may have been elevated by the RIPC stimuli. In initial clinical studies of IPC, higher muscle contents of ATP and CP, and a lower muscle content of lactate were reported following preconditioning (61,49,46). The preservation of ATP and reduction in high-energy phosphate utilization may contribute to the attenuation of muscle fatigue, and thus the increase in power output, through the modulation of K_{ATP} channels. ATP is known to close K_{ATP} channels (5), and a blockade of K_{ATP} channels has been shown to attenuate the decline in tetanic forces in fatigued muscle (21,26). Thus, it is likely that the increase and preservation of ATP produced by preconditioning may contribute to an attenuation of the opening of K_{ATP} channels to an extent so as to reduce muscle fatigue during a series of Wingate tests.

CHAPTER 6

LIMITATIONS AND IMPLICATIONS

There were a few limitations inherent in the present study. As in other previous studies, we could not completely blind our subjects. Although participants remained naïve to the rationale of the experiment, the subjects were likely aware of pressure differences between conditions and could have introduced psychological elements. Additionally, we did not involve a third treatment group, involving ischemic preconditioning of the lower extremity. This type of "local" IPC protocol would have allowed full comparisons of remote and local IPC using identical exercise protocols. Therefore, we cannot fully establish whether one treatment method is preferable over another.

CHAPTER 7

CONCLUSION

In conclusion, a remote ischemic preconditioning protocol applied bilaterally to the upper extremity in healthy, recreationally active individuals increased lower body power output over a series of Wingate anaerobic tests. These results indicate that bilateral RIPC can be an effective means to improve anaerobic power outputs for the purpose of an ergogenic aid. Unilateral RIPC, however, had no effect on any of the performance variables, suggesting that there is a threshold for the amount of target tissue needed to elicit anaerobic performance benefits.

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