The Effect of Ischemic Preconditioning on Repeated Supramaximal Sprints

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Master of Science

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This thesis titled

The Effect of Ischemic Preconditioning on Repeated Supramaximal Sprints

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ABSTRACT

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The Effect of Ischemic Preconditioning on Repeated Supramaximal Sprints

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A single ischemic preconditioning (IPC) treatment can produce acute increases in VO$_2$max that may impact maximal power output. The purpose of this investigation was to determine if IPC will result in improvements in physical performance of a fatiguing task and enhance recovery from that task.

Young, apparently healthy volunteers (n = 24; 22 men and 2 women) completed two testing sessions during which a 30-second maximal cycling task (Wingate test) was performed to quantify muscle fatigue (% reduction in power output) followed by two subsequent 10-second cycling tests to examine the recovery of power output following the fatigue task. Prior to the respective testing sessions, subjects received IPC or a control (CON) intervention. Repeated measures analysis of variance and paired $t$-tests analyses revealed no statistical differences of the treatment intervention on muscle fatigue or the recovery from fatigue.

These data suggest that IPC is not beneficial for increasing anaerobic muscle performance.

Approved: _____________________________________________________________

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CHAPTER 1: INTRODUCTION

Background Information

Recently, an ischemic preconditioning (IPC) protocol using 5-minute increments of restriction/reperfusion for a total of 30 minutes improved the volume of oxygen consumed (VO$_{2}$max) during maximal exercise by 3% coinciding with an increase in power output of 1.6% in a group of healthy men (de Groot, Thijssen, Sanchez, Ellenkamp, & Hopman, 2010). If the IPC protocol does improve VO$_{2}$max, there is potential to clear lactic acid and accelerate recovery of creatine phosphate, which drops as a result of maintaining adenosine tri-phosphate (ATP) levels following the exercise bout.

Significance of Study

The results of this investigation will enhance our knowledge of the use of IPC to improve physical performance of a fatiguing task and to enhance recovery from that task. If IPC proves to enhance performance, these results could be applied to athletic endeavors, rehabilitative techniques, and general fitness improvement programs.

Purpose

The purpose of this investigation was to determine if an acute bout of IPC will result in improvements in physical performance of a fatiguing task and enhance recovery from that task.

Hypothesis

It is hypothesized that an acute IPC treatment will result in less of a decline in peak and mean power output in the first Wingate test as well as subsequent Wingate tests.
Definition of Terms

This investigation uses the following definitions of terms:

*Adenosine tri-phosphate (ATP).* An energy transfer molecule. The phosphate group is removed by breaking a high energy bond, allowing the release of energy. Through the use of specific enzyme systems the body can harvest the released energy from within the bond and use it to perform work.

*Aerobic.* With oxygen present; indicating the biochemical breakdown of nutrients with oxygen.

*Anaerobic.* Without oxygen present; indicating alternate biochemical pathways are utilized than those seen during aerobic metabolism.

*Body composition.* The breakdown of what makes-up the body, i.e., fat, lean muscle, bone and water content.

*Fatigue index.* A mathematical equation used to show the relative amount of decline in power output \[\frac{\text{(peak-min)}}{\text{peak}}} \times 100 = \% \text{ decline}\].

*Ischemia.* Inadequate blood flow; if prolonged, can lead to decreased oxygen tension (hypoxia).

*Ischemic preconditioning.* The act of inducing sub lethal ischemic conditions on cells which triggers them to build up protective measures to better accommodate to ischemic stress.

*Maximal oxygen consumption.* Commonly referred to as VO$_2$ max; the maximum rate at which the body can consume oxygen during maximum exercise.
Percent body fat. The percentage of body mass that is not composed of lean muscle, water, bones or organs.

Power (mean). A mathematical equation used to show the average amount of power output \([\frac{(output\ 1 + output\ 2 + output\ 3…)}{\text{total\ number\ of\ outputs}} = \text{mean output}]\).

Power (minimum). The average of the lowest 5 seconds of power output measured during a power test. Typically at the end of the testing procedure in a group of power outputs.

Power (peak). The greatest power output measured during a power test (power outputs based on 5 second averages). Typically occurs near the beginning of the testing procedure in a group of power outputs.

Rating of perceived exertion. Rating of perceived exertion (RPE) is a psychophysiological assessment of exercise intensity. It uses a chart to measure the whole-body level of exertion experienced by an exercising participant. It is a numerical chart ranging from 6 to 20. A 6 represents a very calm, comfortable state of relaxation while 20 represents the most intense exercise possible and the subject cannot continue.

Revolutions per minute. Revolutions per minute (RPM) is a ratio used in cycling as it is a measurement of the number of times the flywheel makes one full revolution in one minute time.

Supramaximal. An exercise intensity that requires oxygen consumption above the maximal rate.
Assumptions

The assumptions were made that subjects followed the researcher’s instructions during the time spent outside of the laboratory. This included no smoking, caffeine, alcohol, or exercise within 48 hours of testing.

Limitations

One major limitation to the study is the fact that the IPC treatment cannot be blinded. Subjects will distinguish the IPC treatment from the control; however, they will remain naïve to the possible effects of IPC on maximal exercise. Additionally, power outputs throughout all trials will be withheld from the subjects until they have completed the study.

One further limitation to the investigation was that the equipment was limited by a maximum inflation pressure of 300mmHg. As a result, leg occlusive pressure were determined to be either an exact pressure at or below 300mmHg or reported as above 300mmHg.

Delimitations

Due to the study design, cellular mechanisms associated with IPC and maximal exercise will not be measured. The investigation will focus on the effect of IPC on power outputs during repeated sprints in an effort to evaluate the use of the treatment for the enhancement of athletic and rehabilitation performance.
CHAPTER 2: REVIEW OF LITERATURE

Ischemia as it Relates to Exercise

The goal of exercise training, whether for the athlete or an individual in rehabilitation, is to improve physical performance. Recent evidence suggests that creating short bouts of ischemia (inadequate blood flow to a local area) improves aerobic energy production and power output in athletes (de Groot et al., 2010). Interestingly, ischemia is predominantly thought to be detrimental because, if allowed to extend, it can result in hypoxia—inadequate oxygen tension (Kivisaari, Vihersaari, Renvall, & Niinikoski, 1975). Reduced oxygen availability may impede aerobic energy production and a replenishment of stored phosphagens (ATP and creatine phosphate) for repeated bouts of activity (Brooks, Fahey, & Baldwin, 2005). Additionally, the altered metabolism may result in a buildup of byproducts that can disrupt cellular homeostasis—e.g., excess hydrogen ion (Williamson, Schaffer, Ford, & Safer, 1976), free radical formation (Rabl, Khoschsorur, Columbo, Tatzber, & Esterbauer, 1992).

Experimental evidence has demonstrated that skeletal muscle can survive without structural damage following short-term ischemia (Labbe, Lindsay, & Walker, 1987; Huk et al., 1996). Muscular contractions themselves cause transient crushing of vessels, ultimately disrupting blood flow. Illustrated with nuclear magnetic resonance imaging and near-infrared spectroscopy, myoglobin becomes deoxygenated even under light exercise, indicating a drop in cellular PO₂ (Tran et al., 1998). With this evidence, it is expected that daily activities such as walking induces bursts of ischemia that our bodies can cope with successfully.
Adenosine Tri-Phosphate Production

Availability of oxygen largely determines adenosine tri-phosphate (ATP) synthesis (Powers & Howley, 2006), which is an essential component for muscle contraction (Tullson & Terjung, 1991). A limited amount of ATP is available at any given time; therefore, as ATP is being utilized, rapid and continuous maintenance of these concentrations is essential to muscular performance (Tomlin & Wenger, 2001). As ATP consumption increases, immediate rephosphorylation occurs through the creatine kinase reaction (Cain & Davies, 1962). Additionally, the rise in ADP and alterations of other cellular constituents initiate glycolytic flux, which also results in an increase in ATP resynthesis (Powers & Howley, 2006). Following glycolytic degradation of glucose, the product pyruvate follows one of two paths—oxidative phosphorylation or conversion to lactic acid.

Aerobic metabolism is a significant producer of ATP. Although a time consuming process, pyruvate is transported to the mitochondria as a substrate for oxidative phosphorylation where large quantities of ATP are produced through the electron transport chain (Powers & Howley, 2006). As ATP demand increases above the amount that can be produced through aerobic synthesis, such as during maximal exercise, creatine phosphate and anaerobic glycolysis become increasingly more important producers of ATP (Hirvonen, Rehunen, Rusko, & Harkoven, 1987). Anaerobic metabolism can maintain ATP concentrations at very high resynthesis rates, but only for short periods of time. As creatine phosphate stores near depletion and lactic acid accumulates as a by-product of the anaerobic metabolism of pyruvate, ATP resynthesis will be compromised
and maximal exercise performance will decline (Jacobs, Tesch, Bar-Or, Karlsson, & Dotan, 1993). Moreover, during the recovery from an ATP consuming activity, when demands are lower, aerobic metabolism is important as it replenishes anaerobic stores, preparing muscles for the next stress to ATP concentrations (Bogdanis, Nevill, Boobis, & Lakomy, 1996).

**Force Production**

In skeletal muscle, ATP is essential for contraction and the generation of force. Muscle contractions occur at the level of the sarcomere where the myofilaments actin and myosin interact in the presence of ATP to form a cross bridge and produce a power stroke according to the sliding filament theory. The number of cross bridges at any instant dictates how much force is being produced by that muscle (Stone & O’Bryant, 1987). Therefore, exercise producing large amounts of force requires large amounts of ATP.

**Acute and Chronic Ischemia**

Ischemia can be induced through a pneumatic cuff (tourniquet) placed on a particular extremity. Inflating a cuff to a pressure that exceeds systolic blood pressure can reduce PO$_2$ by 11.5\% in as little as 7 minutes (Lebon, Carlier, Brillault-Salvat, & Leroy-Willig, 1998). The acute ischemia can cause changes in metabolism directly as well as indirectly. The ischemia can directly increase glucose uptake, as it is stimulated through hypoxia (Azevedo, Carey, Pories, Morris, & Dohm, 1995). Ischemia could be increasing pyruvate concentrations (Eklof, Neglen, & Thomson, 1980), which will contribute to the activation of pyruvate dehydrogenase (PDH) by increasing substrate availability (Watt, Howlett, Febbraio, Spriet, & Hargreaves, 2001). PDH is an enzyme that catalyzes the
conversion of pyruvate to acetyl Co A, which shifts metabolism to more aerobic sources. The increase in activated PDH can increase carbohydrate metabolism independent of glycolytic activity (Lewandowski & White, 1995) and can significantly delay time to fatigue in skeletal muscle (Platz et al., 2007).

The acute ischemia caused by the occlusive pressure of the tourniquet can also induce reactive hyperemia which is thought to be stimulated by anaerobic metabolites (nitric oxide/adenosine produced by endothelium) common with arterial occlusions longer than 45-seconds (Toth, Pal, Intaglietta, & Johnson, 2007). The reactive hyperemia can result in a state of hyperoxia of 18.9% just 43 seconds after the tourniquet is deflated in young healthy subjects (Schulte, Aschwanden, & Bilecen, 2008).

Acute ischemia induced relatedly may result in chronic physiological adaptations. Takarada et al. (2000) demonstrated the blood flow restricted resistance training technique in elbow flexors using low intensity exercise (30% 1RM) with occlusion and compared it to high intensity exercise (80% 1RM) without occlusion. The results suggested that hypertrophy was similar following 16 weeks of training; however, both groups had significantly greater hypertrophy than the third group which performed the exact same low intensity protocol without occlusion. The exact reason for the hypertrophy following blood flow restriction exercise is not known; however, Takarda and colleagues suggested that performing the exercise with occlusion pressures mimics the metabolism, size principle of fiber recruitment and hormonal responses experienced during high intensity resistance training.
Blood flow restricted exercise has also been shown to elicit improvements in aerobic capacity similar to high intensity exercise. Park et al. (2010) demonstrated that five intervals of 3 minutes walking at 4-6 km/h, resting for 1 minute, 2 times a day, 6 days a week for 2 weeks improved VO$_2$max by 11.6% while the control group did not improve. The researchers attributed the increase in VO$_2$max to an increase in stroke volume of 21.4% and a decrease in heart rate by 15%. These data suggests that the external pressure provided by the tourniquets trained the heart, thus resulting in the increase in aerobic capacity.

Both acute and chronic ischemia imposed as short sessions are reported to be tolerated without many negative consequences (Clark et al., 2010). Problems arise when ischemia is prolonged to the point that there is a complete loss of high energy phosphates which can lead to cell death/necrosis. Researchers recently demonstrated that a prolonged ischemic threat (3 hours) causes injury which may promote acute detrimental effects on skeletal muscle. Likewise, the injury recovery is prolonged when compared to the recovery period needed after injection of a myotoxic solution (Vignaud et al., 2010).

Ischemic Preconditioning

IPC is an example of hormesis, which is defined as an adaptive response of cellular mechanisms to intermittent stress (Mattson, 2008). In 1986, Murry, Jennings, and Reimer demonstrated that the ischemia/reperfusion associated with IPC resulted in protective effects on canine cardiac muscle. The ischemic preconditioning protocol consisted of four, 5-minute bouts of occlusion separated by four, 5-minute bouts of reperfusion. This elicited a protective response that significantly decreased infarct size
when compared to cardiac muscle that did not receive the preconditioning treatment (Murry et al., 1986). The IPC phenomenon has been successful in clinical studies involving smooth muscle found in the liver (Clavien et al., 2003) and lungs (Chen, Li, & Long, 1999), as well as skeletal muscle (Kharbanda et al., 2001). Most recently, interest in IPC has taken a turn towards exercise performance. Although ischemia levels during exercise are minimal compared to the lethal levels used by Murry et al. in 1986, recent publications (de Groot et al., 2010) suggest that performance may be enhanced by IPC, perhaps by some of the following mechanisms.

Ischemic Preconditioning Mechanisms That May Alter Exercise Performance

The effects of temporary, acute ischemia and reperfusion from ischemia–the use of IPC–have been demonstrated to occur quickly (less than 40 minutes) (Murry et al., 1986). Since Murry et al.’s initial research utilizing IPC to protect tissue from subsequent ischemia and reperfusion, research has identified the protective roles of adenosine, bradykinin, opioids and oxygen radicals, which appear to combine to signal the cascade which provides the ischemia/reperfusion protection (Cohen, Baines, & Downey, 2000). The ischemia induced by the tourniquet during IPC tends to stimulate the beginning markers of IPC protection.

Adenosine which is produced rapidly by the endothelium during ischemia (Minamino et al., 1995) is believed to be triggering the reactive hyperemia seen with IPC (Toth et al., 2007). Adenosine acting alone can induce the protective mechanisms of ischemic preconditioning. Bushell, Klenerman, Talyor, Davies, et al. (2002) demonstrated that when rat skeletal muscle was infused with adenosine prior to an
ischemic threat, ATP levels were maintained. The bouts of temporary, acute ischemia performed during IPC may cause a release of adenosine which would help to protect against future bouts of ischemia. It is possible that the adenosine might be derived from the vasculature and not the skeletal muscle itself, because skeletal muscle has small amounts of adenosine relative to smooth muscle (Ronquist et al., 1993). If the vasculature is producing the adenosine which provides the protection associated with IPC, it would be an explanation as to why the protective effects of IPC are generally accepted in cardiac, smooth and skeletal muscle as well as an explanation of why the IPC effects are so rapid.

As discussed, adenosine causes vasodilatation. The same phenomenon occurs as a result of ATP sensitive potassium channels which are subsequently opened by the presence of adenosine. While the ATP sensitive potassium channels are almost fully closed at rest, they are stimulated to open during exercise (hypoxia). This leads to a rise in plasma potassium levels which increases nitric oxide release (Marshall, 2000), causing vasodilatation directly and indirectly. This is in agreement with Kimura et al. (2007) who demonstrated that IPC will increase nitric oxide availability. The channels have been shown to initiate functional sympatholysis (Keller et al., 2004), which is needed to supply active muscles with nutrient rich blood while shunting blood away from less active muscle tissue. Increased vasodilatation at the site of the IPC treatment will increase blood flow to the local muscle beds. ATP sensitive potassium channels are also believed to be involved with regulation of glucose uptake by skeletal muscle independent of insulin (Rodrigo & Standen, 2005). Stimulating the opening of these channels through IPC might
alter metabolism in such a way that additional glucose can be consumed to make ATP, which would lead to a sustained force production. Hopper et al. (2000) suggested that adenosine and ATP sensitive potassium channels play a role in the effectiveness of preconditioning. They showed that when an adenosine-blocker or an ATP sensitive potassium blocker is administered, preconditioning effects declined. However, when adenosine or an ATP sensitive potassium agonists was administered, preconditioning effects increased.

Moreover, the ischemia induced by the IPC protocol might also be stimulating an increase in PDH activity. Calcium release during muscle contraction can stimulate PDH activation; however, calcium can also be activated or maintained in the active state by free ADP and/or pyruvate (Spriet & Heigenhauser, 2002). The increase in PDH activity could have a dramatic impact on increasing aerobic metabolism as a result of IPC. Following IPC, Pang et al. (1995) noted that reactive hyperemia occurred, most likely as a result of adenosine release and other anaerobic metabolites produced during the IPC protocol. Regarding exercise performance, this information suggests that the combination of increasing active PDH, and hyperemia, which might dilute exercise metabolites known to cause fatigue, might ultimately help sustain force production during exercise. This thought is supported by research performed by Gurd et al. (2006). They were able to show that heavy exercise elevates PDH activity, thus speeding up oxygen uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. If IPC can increase PDH activity without taxing the energy stores (as did the heavy exercise), it would be a very beneficial tactic, because it would serve as a low metabolic cost
replacement for warm-up prior to exercise, thus sparing energy stores for later use during competition or rehabilitation.

Exercise with Ischemic Preconditioning

The scientific evidence suggests that when oxygen is present the body is able to produce abundant ATP that provides energy for muscle contractions that create force. During times of exercise, ATP consumption may exceed the rate at which ATP can be produced aerobically. As a result, anaerobic sources are used, but this cannot provide large amounts of ATP for extended periods of time and ultimately there will be a reduced amount of energy for muscle contractions as exercise progresses. The reduction in ATP availability results in a diminished force production. However, the data provided by de Groot et al. (2010) demonstrated that oxygen consumption and power output acutely increased following IPC. Using healthy, well-trained subjects the researchers implemented an IPC trial or a control trial, and then the subjects performed a VO$_2$max test on a cycle ergometer. The study revealed an increase in VO$_2$max of 3% and an increase in maximal power output 1.6% on the IPC trial. The IPC protocol of 30 minutes demonstrated an acute increase in VO$_2$max that is comparable to a month of altitude training (Stray-Gundersen, Chapman, & Levine, 2001). de Groot et al. (2010) believed that the increase in oxygen consumption allowed additional aerobically supplied ATP to be produced, thereby sustaining muscle contractions for a longer period of time. This additional duration could have caused the higher power output. It is interesting to note that de Groot et al. (2010) found no difference in VO$_2$ at submaximal levels. Only when the subjects reached maximal exercise intensities did VO$_2$ increase above the control
group. Pang et al. (1995) demonstrated that IPC might work through ATP-sparring while not compromising force production. de Groot et al. (2010) thought this did not occur in their study, because the muscle consumed the same amount of oxygen at submaximal levels during control and preconditioning trials; thus, the muscle is not more efficient. This would lead one to believe that ATP-sparring could be occurring, but at submaximal levels of exercise, the degree of sparring is so small it was not detected through measuring oxygen consumption. However, the body will extract oxygen to produce ATP to meet the demand of exercise. de Groot et al. (2010) showed that VO$_2$ max increased. This suggests that ATP consumption increased, causing a larger demand to extract oxygen from the blood stream, thus ATP-sparring might not be occurring, but actually consuming ATP at an accelerated rate. Further investigation is needed to explain the ischemic preconditioning phenomenon.

Wingate Testing

The presented literature suggests that IPC alters exercise performance. The literature suggests that alterations in blood flow and metabolism as a result of IPC could be a possible mechanism of increasing ATP production. If this were occurring, it would result in a higher VO$_2$ max as well as higher power outputs just as de Groot et al. (2010) found. In order to determine if IPC has any significant effect on ATP/force production, the Wingate test would be an ideal method of testing. The Wingate was designed to assess anaerobic power using an all-out 30-second sprint on a cycle ergometer using a resistance relative to the individual’s body weight (Bar-Or, 1987). This elicits a maximal effort by participants that requires a high ATP demand in order to produce peak power
output. The Wingate test is commonly used in exercise physiology and has been determined to be a valid and reproducible test (Bar-Or, 1996). Additionally, the Wingate test correlates with cycling performance (Del Coso, & Mora-Rodriguez, 2006), because it mimics situations that rely on bursts of high power intermittently throughout a competition. The 30-second Wingate test utilizes a combination of energy systems during the sprint (Smith & Hill, 1991); however, the test is considered predominately (80%) anaerobic (Beneke, Pollmann, Bleif, Leithauser, & Hutler, 2002).

A 30-second Wingate test elicits fatigue through both peripheral (decrease in force production while EMG activity is unchanged) and central (decrease in EMG activity) mechanisms (Vandewalle, Maton, Bozec, & Guerenbourg, 1991). During the first 10 seconds of maximal exercise energy production is predominately supplied by the phosphocreatine reaction in an effort to maintain adequate ATP concentrations (Cain & Davies, 1962). During the Wingate test, phosphocreatine stores are being depleted, yet simultaneously replenished, just at a much slower rate. Restoration is believed to be done using aerobic metabolism as oxygen consumption is associated with accelerated phosphocreatine replenishment (Bogdanis, et al., 1996). Hebestreit, Mimura and Bar-Or (1993) have shown that most young adult men can recover fully from the test within 10 minutes after completion. If the rest interval is less than 10 minutes between repeated Wingate tests, one can suspect that the subject will still be in a fatigued state and performance will suffer during the subsequent Wingate.

The 30-second sprint shows max peak power outputs 3-5 seconds into the test (Bar-Or, 1996) making a 10-second Wingate test ideal for measuring peak power. As a
result the practical implications were studied in an investigation comparing 10-second versus 30-second sprints performed in athletes. The results show that higher power outputs are achieved using a 10-second Wingate test (Zajac, Jarzabek, & Waskiewicz, 1999). The researchers suggested that a 30-second sprint was very physically demanding so the participants might be “sparring” energy while better effort was put forth during a 10-second sprint. The IPC treatment has been shown to increase VO$_2$max (de Groot et al., 2010); therefore, the body is extracting more oxygen and phosphorylation of ATP should be occurring more rapidly following a fatiguing bout, such as the Wingate test. Repeated Wingate sprints have previously been used to investigate the effects of sodium bicarbonate on hydrogen ion buffering. Lavender and Bird (1989) used repeated 10-second Wingate tests with 50 seconds rest between sprints to provide data of the average power as well as the peak power decline. The work of Lavender and Bird (1989) showed that their protocol was appropriate in causing fatigue, because there was a steady decline in peak power and average power output as the sprints progressed from 1-10. The pyruvate-lactate clearance following fatiguing exercise is believed to be done oxidatively with the help of shuttling lactate from areas of production to areas of consumption (Brooks, Fahey, & Baldwin, 2005). It has been demonstrated that a high aerobic fitness is important in determining the severity of the metabolic response to a high intensity exercise bout (Tomlin & Wenger, 2001). Tomlin (1998) investigated female recreational soccer players using two groups, high VO$_2$max (47.6 ± 3.8 ml/kg/min) and low VO$_2$max (34.4 ± 2.4 ml/kg/min). The subjects were asked to perform ten, 6-second sprints on a cycle ergometer. The high VO$_2$max group consumed significantly more oxygen
compared to their counterparts. Tomlin (1998) suggests that this increase in oxygen consumption allowed the group to have less reliance on anaerobic glycolysis thus less lactic acid production which aided in better power maintenance throughout the ten sprints. These findings suggest that individuals with a higher VO$_2$max will display a better power maintenance during repeated bouts of high intensity exercise.
CHAPTER 3: METHODS

Overview

To determine if an acute bout of IPC would result in improvements in physical performance of a fatiguing task and enhance recovery from that task each participant completed a familiarization trial and two exercise trials. The familiarization trial was at least 48 hours from the two testing trials, and the two testing trials were separated by 4-7 days.

Subject Obligation

Subjects were asked to complete three separate sessions totaling approximately 3 hours of time. The screening tests were set up in sequential order; therefore, if a volunteer did not meet requirements, they would not be required to complete further testing. Those meeting the predetermined criteria were asked to return for Trials 1 (IPC) and 2 (CON). A timeline of the testing trials is shown in Figure 1.

Figure 1. Sequence of testing trials.
Participants

Participants in the investigation were recruited from the campus of Ohio University. Males and females (18-35 years of age) were recruited for the investigation. The research methodology was submitted and approved by the Ohio University Institutional Review Board (IRB#10F020). Female participants were asked to perform Trials 1 and 2 during the same phase of their menstrual cycle. In order to accomplish this, females completed Trial 1 within 1-3 days of initiating menstruation. Trial 2 was then completed no less than 4 days later and no more than 7 days later. To control between genders, the male participants followed the same scheduling, completing Trials 1 and 2 within a 7-day window with at least 4 days separating the trials.

Familiarization

The first session began with introducing the potential participant to the methodology, and to potential risks and benefits of the study using the informed consent document. The form was read by the participant and they had an opportunity to ask the researcher questions for additional clarification. Following the clarification of any questions, the potential participant was asked to sign the Informed Consent Document. The prescreening was then performed using a health history questionnaire. To participate in the investigation, participants could not have had any contraindications to performing maximal exercise such as hypertension, cardiovascular complications, or orthopedic limitations. Participants could not be taking any medications (oral contraceptives, beta-adrenergic bronchodilators, corticosteroids) or ergogenic aids known to affect metabolism (creatine, weight loss supplements). Females must have had a regular
menstrual cycle (predictable duration) and all participants had to be able to refrain from nicotine, caffeine, alcohol and exercise for a 48-hour period. All qualifying participants were then asked to complete further testing consisting of a physical activity questionnaire to rate their current fitness level on a scale of 1-10 (1-4 being “not at all physically fit,” 5-7 being “somewhat physically fit,” 8-10 being “extremely physically fit”). Additionally, determination of leg blood flow occlusive pressure and skin folding to estimate body composition was performed at this time (both described later in greater detail).

Body Composition

Participants were skin folded to estimate body composition. To qualify for inclusion in the investigation, participants needed to be <25% or <30% body fat, men or women, respectively; these are the American College of Sports Medicine endorsed thresholds for establishing obesity (i.e., values greater than standards indicate excess body fat) (Thompson, Gordon, Pescatello, 2010).

During the familiarization trial, participants had skin fold thickness measured at standard 3 site locations using Lange skin fold calipers (Cambridge, MD). On males, the sites consisted of the chest which is a diagonal fold halfway between the anterior axillary line and the nipple, the abdomen which is a vertical fold 2 centimeters to the right side of the umbilicus and the thigh which is a vertical fold halfway between the top of the inguinal crease and the proximal border of the patella. On females, the sites included the triceps which is a vertical fold midway between the acromium process and the olecranon process, the suprailliac which is a diagonal fold following the natural angle of the iliac crest, 1 centimeter above the iliac crest directly below the anterior axillary line and thigh
which is the same fold described for males. All measurements were taken on the right side of the body in duplicate with a minimum of 60 seconds between measurements. If individual site measurements were not within 2 millimeters, a third measurement was taken. The thickness of each skin fold site was averaged and used to calculate body density and ultimately predict body composition of the subject using techniques and calculations suggested by the American College of Sports Medicine (Thompson, Gordon, Pescatello, 2010).

Leg Occlusive Pressure

The IPC protocol utilized in this investigation was identical to the protocol used by de Groot et al. (2010). This protocol used an absolute value of 220mmHg as the restriction pressure exerted by pneumatic cuffs. To determine the pressure exerted relative to each individual, leg occlusive pressure was measured on the familiarization day. Measuring leg occlusive pressure was accomplished by placing inflatable pneumatic pressure cuffs (6 x 83 cm SC5 tourniquet cuff inflated via an E20 Rapid Cuff Inflator, DE Hokanson, Inc., Bellevue, WA) around the upper thigh of both legs and increasing the pressure until the pulse of the posterior tibial artery located posterior to the medial malleolus of the ankle diminished. The equipment was limited by a maximum inflation pressure of 300mmHg. As a result, leg occlusive pressure was determined to be either an exact pressure at or below 300mmHg or reported as above 300mmHg.

Familiarization of Wingate Test

An electronically-braked Lode Cycle Ergometer (Lode BV Excalibur Sport 925900, Groningen, The Netherlands) was used to perform the Wingate Anaerobic Test.
Due to the learning effect of the test (Barfield, Sells, Rowe, & Hannigan-Down, 2002) a familiarization day was used, allowing the participant to experience the exercise protocol. At this time, the participant was fitted to the Lode bike by adjusting the seat and handlebars. The adjustments were recorded and used throughout the remaining trials. Once the participant was mounted on the bike, the warm-up protocol began. The warm up began with 5 minutes of cycling at an intensity of 65 Watts and participant selected RPMs. The next 5 minutes contained two, 5-second Wingate tests with the prescribed load.

The resistance of the cycle ergometer used for the Wingate test was determined as 75 grams per kilogram of the subject’s body mass taken during the familiarization day (Bar-Or, 1996). The resistance remained the same throughout the trials for all subjects. Approximately 15 seconds from the start of the Wingate test, the subjects were instructed to increase the pedal frequency steadily until 2-3 seconds from the test, at this time the subjects sprinted to achieve the highest possible revolutions per minute (RPM). At the start of the test the predetermined resistance was added and the test began. The Wingate tests during the warm up were 5 seconds in duration and separated by 115 seconds of active recovery with 40-watts resistance. After the two Wingate tests were completed, another 115 seconds of unloaded pedaling or rest was performed at the participant’s discretion. At this point, the subject was able to get off the bike and rest for 5 minutes until the exercise protocol began (explained in greater detail later). A diagram of the warm up protocol is displayed in Figure 2.
Figure 2. Warm-up protocol. Subjects completed the protocol prior to the familization trial as well as both testing trials (CON and IPC).

Trial 1 and 2: Ischemic Preconditioning/Control

Trials 1 and 2 served as testing days and were performed in alternating order to reduce an influence of a “learned effect” of the Wingate test. The trials were separated by 4-7 days and were performed at the same time of the day (±2 hours).

The IPC protocol utilized in this investigation (see Figure 3) was intended to induce optimal effects for muscle protection during ischemia when compared with other protocols of varying ischemia/reperfusion ratios ranging from 2-10 minutes of ischemia and 5-15 minutes of reperfusion when performed on rat skeletal muscle (Bushell, Kleinerman, Davies, et al., 2002).

The participants were asked to lie supine for 5 minutes when they arrived at the laboratory. Once the leg cuffs were placed around the proximal thigh of both legs as done during the familiarization trial, one cuff inflated to 220mmHg for a duration of 5 minutes of arterial restriction. At the end of 5 minutes, the cuff pressure was released to allow reperfusion while the other leg cuff was inflated to 220mmHg. Three bouts of
inflation/deflation occurred for each leg, totaling 15 minutes of restriction and 15 minutes of reperfusion. This protocol resulted in one leg finishing 5 minutes closer to the exercise than the other leg. This small amount of elapsed time before exercise was negligible since the early effects of IPC are suggested to last for 2-3 hours (Pasupathy & Homer-Vanniasinkam, 2005). During the control exercise trial, participants remained supine for the duration of 30 minutes wearing the cuffs in the exact location; however they were not inflated (i.e., 0 mmHg). Following the IPC treatment/control, 5 minutes was allowed for participants to transition from the treatment table to the Lode ergometer as they prepared for the exercise protocol.

![Diagram of ischemic preconditioning protocol](image)

**Figure 3.** Ischemic preconditioning protocol. The technique that was used for the IPC protocol is presented as the gray boxes represent arterial occlusion while black boxes represent no arterial occlusion in 5 min increments. Protocol mimics previous work (de Groot et al., 2010).
Exercise Testing

Following the warm-up procedure previously described, the exercise protocol in this investigation utilized a 30-second Wingate test with a 30-second active rest, then a 10-second Wingate test followed by a 200-second active rest and finally ending with another 10-second Wingate test (Figure 4). The active rest in between sprints consisted of the subjects remaining on the cycle ergometer pedaling at a resistance of 40 watts at their own desired frequency. Both the IPC trial and the control trial utilized the same exercise protocol (see Figure 4). The participants were encouraged throughout the test to give great effort, because less than maximal efforts would lead to poor results. With the resistance and RPMs, the Lode ergometer calculated power outputs in watts. Peak power, mean power and fatigue index were analyzed for all trials.

![Exercise protocol diagram](image)

*Figure 4. Exercise protocol. This protocol was utilized during the familiarization trial as well as both testing trials (CON and IPC).*
In this test peak power, mean power, minimum power and fatigue index were calculated. Peak power is determined by averaging the highest 5 seconds of wattage produced by the participant, which usually occurs during the first 5 seconds of the test. Mean power is the average of all the wattages produced over a designated period of time. Minimum power is determined by averaging the lowest 5 seconds of the test. Fatigue index is a calculation used to determine the fatigue induced by the Wingate test. To calculate the fatigue index one must have the peak power and minimum power produced during the test. The difference between the peak power and the minimum power was divided by the peak power. This revealed a decimal which was multiplied by 100 to create a percentage which was the fatigue index. The percentage is considered the amount of watts that the participant was unable to maintain throughout the Wingate test.

Statistical Analysis

To perform the statistical analysis SPSS 17.0 was utilized. All data were recorded as mean ± standard deviation. The initial 30-second Wingate was analyzed to determine a peak power, mean power, minimum power and fatigue index for that particular test. The first 10 seconds of the 30-second Wingate was used to calculate peak power, mean power, minimum power and fatigue index (referred to as peak power$_{10}$, mean power$_{10}$, minimum power$_{10}$ and fatigue power$_{10}$) of the first Wingate, which was then compared the same values for the subsequent Wingate tests.

To analyze the Wingate tests on the CON day versus the IPC day, multiple statistical tests were used. In order to evaluate the initial Wingate values, a $t$-test was used
between the CON and IPC days for all values. In order to evaluate the power outputs and fatigue indexes of the repeated Wingate tests, a 2x3 ANOVA was utilized.

A power calculation was performed to determine the statistical power for the sample size in the present project (n = 24). Specifically, based on the assumption of a repeated measures ANOVA with n = 24 and alpha set to 0.05 and a correlation between measures of 0.89 we have power equal to 0.50 to detect a small effect with respect to the IPC intervention (eta-squared = 0.09). All power calculations were performed with G*Power 3.0.3 (Universität Kiel, Germany).
CHAPTER 4: RESULTS

A total of 27 participants from Ohio University Athens campus were recruited as volunteers for this investigation. Three of the subjects were unable to complete the exercise trials in the time allotted; as a result, 24 subjects (male, n = 22; female, n = 2) completed all trials and requirements of the research investigation. Subject characteristics are presented in Table 1.

Table 1

Subject Characteristics

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
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<td>± 3</td>
<td>20-31</td>
</tr>
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<td>Height (cm)</td>
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<td>± 177.91</td>
<td>163.20-191.77</td>
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<td>Weight (kg)</td>
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<td>± 12.81</td>
<td>52.41-110.00</td>
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<td>± 6.11</td>
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<tr>
<td>Fat Mass (kg)</td>
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<tr>
<td>Fat-Free Mass (kg)</td>
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<td>± 10.40</td>
<td>61.99-85.80</td>
</tr>
<tr>
<td>Self Reported Physical Fitness*</td>
<td>8</td>
<td>± 1</td>
<td>5-10</td>
</tr>
</tbody>
</table>

*scale 1-10; 1=not at all physically fit, 10=extremely physically fit
The variables peak power, mean power, minimum power and fatigue index of the initial 30-second Wingate for the CON trial were compared to the IPC trial using a paired $t$-test. Peak power (CON = 1111.56 ± 248.50 watts, IPC = 1104 ± 238.28 watts) was not significantly different ($p = .480$); group means are illustrated in Figure 5. Mean power (CON = 762.03 ± 118.92 watts, IPC = 766.82 ± 121.09 watts) was not significantly different ($p = .406$); the group means are presented in Figure 6. Minimum power (CON = 563.01 ± 86.72 watts, IPC = 541.60 ± 77.80 watts) revealed no significant difference ($p = .577$); group means are presented in Figure 7. Fatigue index (CON = 50.08% ± 10.90%, IPC = 49.53% ± 8.86%) revealed no significant difference ($p = .670$) presented in Figure 8.

![Peak Power](image)

Figure 5. Peak power outputs during initial 30-second Wingate test. (CON = 1111.56 ± 248.50 watts, IPC = 1104 ± 238.28 watts.)
Figure 6. Mean power outputs during initial 30-second Wingate test. (CON = 762.03 ± 118.92 watts, IPC = 766.82 ± 121.09 watts.)

Figure 7. Minimum power outputs during initial 30-second Wingate test. (CON = 563.01 ± 86.72 watts, IPC = 541.60 ± 77.80 watts.)
To compare across all three Wingate tests for subsequent analyses, the first 10 seconds of this first 30-second Wingate test were used to recalculate peak power, mean power, minimum power and fatigue index (identified as peak power_{10}, mean power_{10}, minimum power_{10} and fatigue index_{10}, respectively). Each variable was compared to the second and third sprint using a 2x3 (groups x tests) analysis of variance (ANOVA).

The repeated measures ANOVA revealed that the peak power_{10} of the CON versus IPC trials was not significantly different for the intervention \((p = .742)\) or the intervention*time interaction \((p = .442)\); the group means are presented in Figure 9.

There was, however, a significant main effect for time \((p = 0.001)\) with peak power_{10} of 1,107.91 watts vs. 915.43 watts vs. 1,065.39 for the first, second, third test, respectively.
Post hoc analysis using Least Significant Difference (LSD) illustrated peak power$_{10}$ 1 > 2 ($p = .001$), 1 > 3 ($p = .009$) and 3 > 2 ($p = .001$).

![Peak Power$_{10}$](image.png)

*Figure 9. Peak power$_{10}$ outputs during three, 10-second Wingate tests.* Wingate 1 > 2, 3; **Wingate 3 > 2.

Further analysis revealed that the mean power$_{10}$ of the CON versus IPC trials were not significantly different for the intervention ($p = .356$) or the intervention*time interaction ($p = .131$); the group means are presented in Figure 10. There was, however, a significant main effect for time ($p = 0.001$), with mean power$_{10}$ 994.51 watts vs. 752.99 watts vs. 914.40 watts for the first, second, third test, respectively. Post hoc analysis using LSD illustrated mean power$_{10}$ 1 > 2 ($p = .001$), 1 > 3 ($p = .001$) and 3 > 2 ($p = .001$).
Figure 10. Mean power_{10} outputs during three, 10-second Wingate tests. * Wingate 1 > 2, 3; ** Wingate 3 > 2.

Analysis of minimum power_{10} between CON and IPC trials also revealed no significant difference for the intervention (p = .404) or the intervention*time interaction (p = .218); the group means are presented in Figure 11. There was, however, a significant main effect for time (p = 0.001), with minimum power_{10} 904.13 watts vs. 623.60 watts vs. 807.37 watts for the first, second, third test, respectively. Post hoc analysis using LSD illustrated minimum power_{10} 1 > 2 (p = .001), 1 > 3 (p = .001) and 3 > 2 (p = .001).
Figure 11. Minimum power_10 outputs during three, 10-second Wingate tests. * Wingate 1 > 2, 3; ** Wingate 3 > 2.

Analysis of the fatigue index_10 across the three Wingate tests revealed no significant difference for intervention (p = .332) or intervention*time interaction (p = .849) between the CON and IPC trials; the group means are presented in Figure 12. There was, however, a significant main effect over time (p = 0.001) with fatigue index_10 17.78% vs. 31.00% vs. 23.20% for the first, second, third test, respectively. Post hoc analysis using LSD illustrated fatigue index_10 1 < 2 (p = .001), 1 < 3 (p = .001) and 3 < 2 (p = .001).
Figure 12. Fatigue index$_{10}$ during the three, 10-second Wingate tests. * Wingate 1 $<$ 2, 3; ** Wingate 3 $<$ 2.

Leg occlusive pressures were recorded during the familiarization day. The leg occlusive pressures were all reported as $> 300$mmHg with the exception of one subject (270 mmHg, right and left legs). Therefore, the researchers were unable to determine the relative occlusive pressure for each subject elicited by the absolute pressure of 220mmHg during the IPC protocol. Testing days were performed in alternating fashion to achieve equal amount of subjects to start with the IPC trial ($n = 12$) and the CON trial ($n = 12$). The order of the testing days showed no effect on power output: The group means were not significantly different when compared across the three Wingate tests, nor was the fatigue index significantly different for the two groups.
CHAPTER 5: DISCUSSION

The purpose of this investigation was to determine if IPC would result in improvements in physical performance of a fatiguing task and enhance recovery from that task. In this investigation, IPC was used before a 30-second Wingate test, and repeated 10-second Wingate tests to determine its effect on power outputs. No significant differences in power outputs were found between IPC and CON trials in the initial 30-second Wingate or the repeated 10-second Wingate tests. The current investigation did find differences in peak power, mean power, minimum power and fatigue index between Wingate tests 1, 2, and 3, demonstrating that the protocol successfully induced fatigue.

Following the initial Wingate test in the current investigation, it was hypothesized that greater aerobic capacity, postulated to result from IPC, would improve the recovery and translate into greater performance on the repeated Wingate tests. In both the CON and IPC trials, the protocol did induce fatigue, as the third Wingate tests were significantly lower in mean power than the initial tests (996.80 vs. 901.87 watts, 992.21 vs. 926.93 watts, CON vs. IPC, respectively). However, while not significantly different, the third Wingate of the IPC trial was on average 25 watts higher than the CON trial. Moreover, this translated into a greater recovery of 3.19% after the IPC treatment.

Previous research has demonstrated IPC to increase VO2max (de Groot et al., 2010), possibly through pathways that aid in aerobic metabolism. The IPC protocol is effective in inducing ischemia (Lebon et al., 1998) and, therefore, evidence garnered from ischemic literature relates strongly to this investigation. One particularly important
point is that ischemia itself generates excess hydrogen ion concentrations (Neglén, Carlsson, Eklöf, Gustafson, & Thomson, 1979). The altered pH and the negative impact that the hydrogen ions might impart on the skeletal muscle cross bridge formation (Lavender & Bird, 1989) may result in a reduction in power output.

In addition, reactive hyperemia (increased blood flow) is a result of the IPC protocol (Toth et al., 2007) and, in and of itself, is partially responsible for the protection from subsequent ischemia (Murry et al., 1986) and potentially increased performance (de Groot et al., 2010). Anecdotally, subjects often reported their legs felt “heavy” following the IPC protocol. If blood pooling in the legs was experienced throughout this protocol, it may have contributed to the current investigation’s inability to demonstrate improved power outputs with IPC. In other words, the “heavy” legs might have led to a struggle to increase and/or maintain RPMs and, therefore, the calculated power outputs. However, no differences were found between the initial RPMs between treatments.

In addition, ischemia induces a decrease in PDH activity (Kobayashi & Neely, 1983), but increases pyruvate, lactate, and hydrogen ion concentrations (Eklof et al., 1980). These altered metabolic products and modulators have been demonstrated to increase PDH activity as a result of increasing substrate availability via glycolytic flux (Watt et al., 2001). Therefore, during a period of reduced oxygen availability the tissue will not rely heavily on the PDH reaction (to generate substrate for the Krebs Cycle) and decrease the aerobic contribution to energy production (Greenhaff & Timmons, 1998). With the cessation of IPC, and the ensuing reactive hyperemia, PDH activity becomes increasingly important to contribute substrate for aerobic metabolism. This increase in
aerobic metabolism works to directly replenish ATP and creatine phosphate, which plays a critical part in preparing for the subsequent Wingate tests (Bogdanis et al., 1996).

Past research has demonstrated that the percentage of total PDH in the active form at any given time roughly matches energy demands and correlates well with the relative aerobic power output (Howlett et al., 1998). The failure of the current investigation to identify differences in power output in the initial or repeated Wingate tests might be due to the protocol itself. Both the IPC and CON trials utilized a 10-minute low intensity cycling activity interspersed with 5-second all-out sprints. Parolin et al. (1999) demonstrated that PDH activity rapidly increased within the first 6 seconds of a cycle sprint, and reached maximum in 15 seconds; therefore, it is likely the warm-up protocol in this investigation stimulated PDH activation, thus limiting any additional power output attributed to the total PDH activation potentially induced by the IPC treatment. However, while de Groot et al. (2010) increased VO\textsubscript{2}max using the same IPC treatment, this occurred following an extensive submaximal phase of exercise (12 minutes of a ramp protocol). During this time, subjects would have been steadily increasing PDH activity due to the stress of the exercise, not necessarily as a result of the IPC intervention. This suggests that the PDH activation may not be a major contributor to the increase in VO\textsubscript{2}max associated with IPC.

The protocol in this investigation successfully caused fatigue and monitored recovery. Extending or shortening the active recovery between the Wingate tests might result in additional metabolic, neurological or other physiological explanations for recovery from the fatiguing task beyond that of any possible contribution of the IPC
treatment itself. If the active rest intervals were shortened, subjects who were primarily anaerobic by nature might not recover enough to see a difference between the repeated Wingate tests. Had the active rest between the sprints been extended, subjects who were aerobic by nature might recover fully, thus not demonstrating any effects of IPC. Therefore, the lack of differences in power outputs may lie in the Wingate test itself.

While it is possible that subjects in this investigation increased their VO$_2$max similarly to de Groot et al. (2010), the 30-second Wingate test did not allow for a great enough aerobic contribution to total energy demand in this short period. Smith and Hill (1991) analyzed the aerobic and anaerobic contributions to a 30-second Wingate test in 5-second increments. It was determined that during the test aerobic contributions do not exceed 16%. It is likely that if the test were longer than 30 seconds, the IPC protocol would have improved power outputs. Furthermore, failure of the data to be significantly different during the repeated Wingate tests might be explained by de Groot et al. (2010) who reported that there were no changes in VO$_2$ at submaximal levels, only at maximal levels.

In the current investigation, the VO$_2$ consumed during the recovery is unknown. It is possible that the intensity of 40 watts during the active recovery did not elicit a VO$_2$ (aerobic metabolism) high enough to reap the benefits of the IPC treatment; thus, any effects of the treatment would not be detected.

Lastly, the lack of effect IPC had on power outputs could relate to aerobic and/or anaerobic training status of the subjects used in this study. While not directly measured in this investigation, subjects self-reported their physical fitness levels (8±1, on scale 1-10). The physical fitness questionnaire revealed that the sample reported to have either
“somewhat high fitness levels” or “extremely high fitness levels.” It is possible that the IPC treatment had a diminished effect on individuals with high fitness levels. In contrast, however, de Groot et al. (2010) illustrated the success of this same IPC protocol in “well-trained” individuals (VO\textsubscript{2 max} avg. = 56.8ml/kg/min).

Moreover, statistical analyses for this investigation included two female participants. Because of the low recruitment of this gender, all statistical analyses were re-analyzed after excluding females for absolute and relative (watts/kilogram of body mass) power outputs. Because no differences were found, these data were included in the data set. In this investigation, female participation was scheduled around their menstrual phases. Specifically, because of the influence on power output both trials occurred during the same menstrual phase (Middleton & Wenger, 2005). In order to determine a gender effect, although not part of this investigation, a larger population of female participants would need to be recruited.

One further explanation for a lack of effect of the IPC treatment on power output and recovery from fatigue was the failure to use relative IPC pressure proportional to the subject’s own leg occlusive pressure. The pressure used was an absolute pressure of 220mmHg, and, although this resulted in significant increases in VO\textsubscript{2 max} for de Groot et al. (2010), this may have limited the effectiveness of IPC in this investigation.

Future research investigating the effects of IPC on exercise performance should first confirm that that IPC treatment improves VO\textsubscript{2 max}. Additionally, investigations using IPC treatment to improve performance and stave off fatigue should use exercise protocols that maintain intensities close to VO\textsubscript{2 max} for longer durations than used in this
investigation. Longer durations of exercise at these higher intensities might tease out differences in power outputs as a result of IPC treatment. And lastly, it is important to suggest that the IPC treatment itself may not influence anaerobic ATP production or performance.

In conclusion, the current investigation demonstrated that an acute bout of IPC had no effect on the power outputs or fatigue index of a single 30-second Wingate test or repeated 10-second Wingate tests. While a small difference may lie in the recovery from the fatiguing task when IPC treatment was provided, it did not reach statistical significance. The data suggest that an acute bout of IPC treatment has no effect on maximal power outputs in anaerobic tests in individuals with higher self-reported fitness.
REFERENCES


APPENDIX A: INFORMED CONSENT DOCUMENT

Ohio University Consent Form

The effects of ischemic preconditioning on repeated supramaximal sprints

Researcher: Marcus W. Barr
Advisor: Dr. Michael Kushnick

You are volunteering to participate in this research investigation. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

EXPLANATION OF STUDY

Purpose of the research
The purpose of this study is to examine the effects of ischemic preconditioning, temporary reducing blood flow to non-working muscles (legs), on power output of repeated maximal cycling sprints. Ischemic preconditioning is a novel technique and has been shown to alter other aspects of exercise performance; but further research is needed to understand if this intervention alters this specific aspect of performance.

Subject Obligation
Total obligation for those that qualify will be 3 separate sessions totaling approximately 3 hours.

Session 1 (pre-screening questionnaire, preparation and familiarization):
You will be asked to participate in the informed consent process (including reading and acknowledging the details and methodology of this study). In addition, you will complete a pre-screening health history questionnaire. To participate in the investigation you must be between the ages of 18-35 years and not have high blood pressure (less than 140mmHg systolic and less than 90 mmHg diastolic at rest). Furthermore, you must not be pregnant or taking oral contraceptives (birth control), have any orthopedic limitations that would prohibit your ability to complete the experiment, not have known bleeding disorders (including sickle cell anemia or blood clotting problems, etc.) or any
contraindications to performing maximal exercise (including, but not limited to unstable angina, known or suspected cardiac arrhythmias, recent infections) and you must not be taking any medications or supplements known to affect metabolism. Men must be less than 25% and women less than 30% body fat to participate – as these are the current endorsed guidelines for establishing the lack of excess body fat (i.e. not obese) – as determined in the next step.

If you meet the above criteria, you will have your body composition analyzed utilizing skin fold calipers. This will be performed by measuring the thickness of adipose tissue (fat tissue) at three locations (Men: Chest- between armpit and nipple, Abdomen- to the side of the belly button, Thigh- between knee and hip; Women: Triceps-back of upper arm between elbow and shoulder, Suprailium- slightly above hip bone, Thigh- between knee and hip). These values will be used to calculate body density which is used to calculate body composition (% body fat) as suggested by the American College of Sports Medicine.

Qualified participants will have a blood pressure cuff placed around their upper leg allowing the researcher to take your leg blood pressure. Next, you will be able to become familiar with the exercise protocol. You must not have performed exercise (physical activity above your daily routine) and have refrained from smoking, caffeine, and alcohol for at least 48 hours prior to this session. In this session you will be familiarized with the protocols and the equipment that will be used during the investigation. The familiarization session will be described in the “exercise protocol” paragraph below (but no ischemic preconditioning will precede the exercise).

**Session 2 or 3 (IPC and Control)**

You must drink fluids (water, juice, etc.) to ensure that you are hydrated for 24 hours prior to experimental sessions, as well as maintaining your hydration for 48 hours following the sessions. You must not have performed exercise (physical activity above your daily routine) and have refrained from smoking, caffeine, and alcohol for at least 48 hours prior to sessions 2 and 3.

Participation of women – As menstrual phase may influence the results of this investigation, women who qualify for participation in this investigation will be required to identify their previous menstrual cycle (menses; for planning purposes). These qualified women will complete Session 2 on day 1, 2, 3 of initiation of their current menstrual cycle (menses) and complete Session 3 no less than 4 days after Session 2, but no more than 7 days later.
Men will be required to complete Session 3 no less than 4 days after Session 2, but no more than 7 days later.

Sessions 2 and 3 will be randomly assigned as either ischemic preconditioning (IPC) or control trials. In the IPC trial an inflatable pneumatic pressure cuffs (6 x 83 cm tourniquet cuff inflated via a rapid cuff inflator) will be placed around the upper thigh of both legs. The cuff on one leg will inflate to 220mmHg for duration of 5 minutes of arterial occlusion. At the end of 5 minutes, the pressure is released to allow reperfusion while the other leg cuff is inflated to 220mmHg. All told, three cycles of inflation/deflation will occur for each leg, totaling 15 minutes of occlusion and 15 minutes reperfusion for each leg. During the control trial, you will lie supine for 30 minutes without any ischemic preconditioning treatment but have the cuffs in place (not inflated/0mmHg).

Following the above (IPC or control) a brief warm up and rest period will be performed. This will consist of 10 minutes of low intensity cycling with two 5-second maximal sprints interspersed. You will then sit in a chair for 5 minutes until the testing begins.

For the testing you will be on the cycle ready to perform five maximal sprints. A countdown of “10, 9, 8…” will be given. At “10” you will be encouraged to begin increasing your pedal frequency (against no resistance) so that at “3” your pedal frequency will have reached its peak. At “0” the predetermined resistance (.075g per kg of your body weight) will be applied to the cycle signaling the start of the 30 second test. At the end of the 30 seconds, the wingate resistance will be removed. You will stay on the cycle, pedaling to recover and after 30 seconds will begin a 10 second sprint. You will stay on the cycle for another 200 seconds, pedaling to recover until performing the final 10 second sprint.
Risks and Discomforts
All procedures have been designed and will be implemented in order to minimize the potential risks to you. All research investigators and technicians will be CPR and First Aid certified and familiar with the safety plan that has been implemented in this laboratory – if an incident is severe the investigators may activate the emergency medical system (ambulance).

As with all exercise there are inherent risks of performing this experiment. These risks include, but are not limited to, cardiac emergencies and muscle/orthopedic problems including rhabdomyolysis and compartment syndrome which may also be associated with blood occlusion. Rhabdomyolysis is the breakdown of muscle fiber resulting in the release its contents into the bloodstream which are often exemplified in the body as abnormal urine color (example – dark, red, or cola colored), unexplained general muscle weakness (often localized in the affected muscles), stiffness or aching. Compartment syndrome is the compression of nerves, muscle, and blood vessels within a closed space in the body. Increased pressure within a closed space can lead to tissue death in the local area, and potentially the loss of limbs. Signs or symptoms of Compartment Syndrome include:

- Deep, constant, and severe pain
- Decreased sensation
- Numbness or “Pins and Needles” sensation in the area
- Pale or shiny skin
- Weakness

To minimize risks, you will complete a health history questionnaire – disclosing pertinent medical information and medications/supplements you are currently taking which may exclude you from this study. Furthermore, it is necessary that you are adequately hydrated 24 hours prior to and throughout the entire investigative period.

Further, the cuffs used for the IPC protocol is likely to cause mild discomfort to some individuals. The sensation will be similar to having a blood pressure taken; only the cuffs will be around the legs and the sensation will last for five minutes. There is a risk of swelling and bruising when IPC is performed.

If you experience any of these symptoms, please do not hesitate contact myself and/or Dr. Kushnick at any time of the day or night using the information listed below and/or seek medical attention.

You will be aware of the purpose and procedure for each test that will be performed before it begins and will be made aware of what is expected of you throughout the investigation. You are encouraged to ask questions during the testing and/or to contact
the researcher at any time during the course of this investigation with questions, comments, or concerns and should be aware that you are under no commitment to continue if unwilling or unable to continue. Your participation is completely voluntary.

Benefits
Anticipated benefits to you include exposure to the experimental protocol, acquisition of data, experience in an exercise science laboratory and knowledge of your body composition (% body fat).

Confidentiality and Records
All data will be in possession of the principal investigator or will be kept in Dr. Michael Kushnick’s office in a locked file. You will receive subject identification and only the investigator will be able to identify your records. A code key will be developed to match each subject’s name with their subject number. This key will be destroyed after data collection is complete and you and other subjects have been provided with your individual results. The data will be compiled and analyzed with only group data being used for dissemination. After the data is complied and the subjects are provided with their individual results the master list will be destroyed.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with:

* Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research;
* Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU;

Contact Information
If you have any questions regarding this study or experience any problems at any time, the researchers will be available for assistance. Please contact Marc Barr via phone (740-416-0542) or email (mb714205@ohio.edu). You may also contact Dr. Michael Kushnick via phone (740-593-0496) or email (kushnick@ohio.edu).

If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

By signing below, you are agreeing that:

- you have read this consent form (or it has been read to you) and have been given the opportunity to ask questions and have them answered
- you have been informed of potential risks and they have been explained to your satisfaction.
- you understand Ohio University has no funds set aside for any injuries you might receive as a result of participating in this study.
• you are 18 years of age or older
• your participation in this research is completely voluntary
• you may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you and you will not lose any benefits to which you are otherwise entitled.

Signature_________________________________________ Date___________

Printed Name_________________________________________

Version Date: 03/04/11
APPENDIX B: HEALTH HISTORY QUESTIONNAIRE

HEALTH HISTORY

Age:  Sex:  Male  Female

If applicable, regular menstrual cycle (consistent # of days)?   Yes  No

Approximate length of menstrual cycle (# of days)? ________days

Please indicate whether any of the following apply to you. If so, please place a check in the blank beside the appropriate item. Thank you.

___________ Hypertension or high blood pressure

___________ A personal OR family history of heart problems or heart disease

___________ Diabetes

___________ Orthopedic problems

___________ Cigarette smoking or other regular use of tobacco products

___________ Asthma or other chronic respiratory problems

___________ Any other medical or health problems not listed above. (Provide details below.)

________________________________________________________________________
________________________________________________________________________

List any prescription medications, vitamin/nutritional supplements or over-the-counter medicines you routinely take or have taken in the last five days (including dietary/nutritional supplements, herbal remedies, cold or allergy medications, antibiotics, migraine/headache medicines, aspirin, ibuprofen, birth control pills, etc.)

________________________________________________________________________
________________________________________________________________________

Code: ______________________
The effect of ischemic preconditioning on repeated supramaximal sprints

I certify that the information that I provided on the health history form of the above state research project was true, accurate and complete to the best of my knowledge.

Signature _____________________________

Date _____________
APPENDIX C: PHYSICAL ACTIVITY QUESTIONNAIRE

Physical Activity Questionnaire

1. In general, compared to other persons your age, rate how physically fit you are:

1  2  3  4  5  6  7  8  9  10
Not at All Physically Fit  Somewhat Physically Fit  Extremely Physically Fit

2. Including your normal work, daily responsibilities (including school), and exercise (physical activity) how many times per week do you experience increased breathing, increased heart rate and/or sweating for at least 30 minutes (such as brisk walking, cycling, swimming, jogging, aerobic dance, stair climbing, rowing, basketball, racquetball or vigorous yard work)?

☐ Seldom or Never  ☐ Less than 1 time per week
☐ 1-2 times per week  ☐ 3-4 times per week
☐ 5 or more times per week

3. How long have you exercised or played sports regularly?

4. Have you participated in physical activity in the past (>1 year ago)?

Code: ______________