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Effects of Ischemic Preconditioning on Exercise Economy

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EFFECTS OF ISCHEMIC PRECONDITIONING ON EXERCISE ECONOMY

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Nutrition, Exercise, and Food Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

EFFECTS OF ISCHEMIC PRECONDITIONING ON EXERCISE ECONOMY

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Ischemic preconditioning (IPC) is the application of small bouts of ischemia followed by reperfusion. IPC has shown to enhance exercise performance in varied sports like 5K running, cycling and swimming. However, effect of ischemic preconditioning on running economy have not be investigated so far. We proposed that bilateral ischemic preconditioning will improve running economy in young healthy people. Methods: The study was a randomized single- blinded crossover study. Nineteen men and women were included in the study. Each participant underwent a sham and IPC trial using high pressure cuff (EC20 rapid cuff inflator, DE Hokanson, Inc.) followed by running economy trial (Parvo Medics, TrueOne 2400) of 15 minutes. The sham or IPC trial consisted of 3X 5 min of bilateral leg ischemic at pressure of 15 mm of Hg (sham) or 220 mm of Hg (IPC). Each 5 min stage of bilateral leg ischemia was followed by 5 minutes of reperfusion which was followed by 3X 5 min of submaximal treadmill running. Results The data was completed and analyzed for 18 subjects. The mean age of participants was 27+7 years with BMI for all subjects was 24.6 ± 3.0 kg/m^2. A two way ANOVA was used to assess interaction in running economy (i.e., steady-state oxygen consumption, respiratory exchange ratio, heart rate) in the absence and presence of IPC. In the sham running trial, oxygen consumption progressively increased 22% ($P<0.001$) from 34.1 (stage 1) to 41.6 ml/kg/min (stage 3) and 20% ($P<0.001$) from 34.3 (stage 1) to 41.4 ml/kg/min (stage 3) in the IPC running trial. There was no time X trial interaction in

steady-state oxygen. Running economy showed no significant change during the sham running trial, (P=0.232) from 201.6 (stage 1) to 204.0 ml/kg/km (stage 3). The same trend in running economy was observed during the IPC trial (P=0.129; from 202.8 to 203.1 ml/kg/km). There was no time X trial interaction (P=0.647) in running economy. Additionally heart rate showed a general increase from 152±9 (stage 1) to174±6 (stage 3) for the sham trials and 151±11 (stage 1) to 174±8 (stage 3) for IPC trials. We did not find significant differences in heart rate during sham and IPC trials (P= 0.999). Conclusion: The present study did not supported our hypothesis that remote IPC can improve running economy.

1: INTRODUCTION

When the blood supply to the heart is restored after a prolonged period of ischemia, as for example with the use of thrombolytic therapy or primary percutaneous tissue injury intervention to treat an acute myocardial infarction, reperfusion causes tissue injury. This process is referred to as myocardial ischemic/reperfusion (I/R) injury which can lead to cardiac arrhythmias, ventricular dysfunction and even myocardial cell death (Luca, Liuni, McLaughlin, Gori, & Parker, 2013). As a result, reperfusion injury is thought to adversely contribute to clinical outcomes after myocardial infarction, particularly by increasing the severity of tissue damage and rate of recurrent thrombotic events despite optimal reperfusion therapy. The severity of reperfusion injury depends on the duration of ischemia. Short bouts of ischemia (1-5 min) can cause reversible cell dysfunction and electrical disturbances in the cells. Ischemia beyond duration of 5 min elicits more severe cell damage and can lead to tissue death (Hausenloy & Yellon, 2008; [1].

The mechanisms underlying I/R injury are not fully understood. Due to the lack of oxygen and inability to meet aerobic energy requirements there is a depletion of cellular ATP. The ischemia leads to increased anaerobic metabolism for energy production which decreases the intracellular pH. In addition, the lack of ATP inhibits sodium potassium ATPase and ATP-dependent calcium reuptake in sarcoplasmic reticulum. This leads to an increase in calcium concentrations which in turn results in myocardial calcium overload. Restoration of the blood flow reinstates the normal cellular functions and ATP generation; however with more extensive ischemia leads to mitochondrial membrane destabilization (Tapuria et al., 2008).There is more production of reactive oxygen species (ROS) due to activation of xanthine oxidase and related oxidative mechanisms. The

hydroxyl radicals produced as a result of dissociation of the ROS result in higher damage to cell membranes and cellular structure during reperfusion injury. So far, there is no known clinical intervention to prevent or reverse the damage caused by reperfusion injury (Sanada, Komuro, & Kitakaze, 2011). However, a promising strategy is to exploit the inherent cardio protective properties of the myocardium by conditioning it to become more tolerant to I/R injury, a property known as ischemic preconditioning (IPC).

IPC is a type of conditioning stimulus applied before, during, or after a sustained ischemic episode. The conditioning stimulus can also be applied remotely of the target organ to protect it against I/R injury, referred to as remote preconditioning. In this respect, subjecting a distant organ to brief sublethal bouts of ischemia followed by brief reperfusion protects the target organ against I/R injury. Experimental models have shown that preconditioning can protect many organs from the damage caused by I/R injury including the heart, kidney, brain, lungs and skeletal muscles (Zhou et al., 2007). One of the main mechanisms by which IPC is protective involves its capacity to prevent reductions in the blood flow that accompanies endothelial and myocardial I/R damage.

Exercise economy is an important component that plays a central role in endurance exercise performance. An economical athlete will consume less oxygen for a given steady-state submaximal exercise workload than a less economical athlete, and as a result, will show significant performance benefits, particularly at long distance events by expending less energy. Improvements in exercise economy following IPC may contribute, in part, to enhanced exercise performance. Recently it was shown that IPC prevents reduction in blood flow after strenuous exercise and improves swimming, cycling and running performance (Bailey et al., 2012; De Groot et al. 2010; Clevidence et al. 2012;

Crisafulli et al., 2011). The reason to explain these performance benefits with IPC are not fully understood. Moreover, to date, there is little scientific insight into whether remote IPC improves exercise economy.

1.1   : STATEMENT OF PROBLEM

Little is known about the effect of remote IPC on exercise performance and a limited number of studies have been published. Moreover, the effect of remote IPC on running economy has not been investigated so far to our knowledge. In the light of previous research it is important to determine the potential beneficial effects of remote IPC on running economy.

1.2   : SPECIFIC AIM

Specific Aim: To determine the effects of remote IPC on runningeconomy.

Hypothesis: We hypothesize that remote IPC will improve runningeconomy.

1.3 : DEFINITION OF TERMS

Ischemia-Reperfusion Injury: Cellular injury caused by the rapid restoration of bloodflow following a sustained period of ischemia.

Ischemic Preconditioning: Ischemic preconditioning is a technique which protects the heart against reperfusion injury by exposing the heart to nonlethal intermittent periods of ischemic bouts prior to prolonged ischemia.

*Remote Ischemic Preconditioning***:** Remote ischemic preconditioning refers to application of small ischemic bouts at regular interval which when applied to one organ confers preconditioning against reperfusion injury to a distant organ.

Exercise Economy: The amount of energy (oxygen consumed) to run at particular velocity.

Experimental Design

Randomized, single-blind cross-over study

1.4 : INDEPENDENT VARIABLE

Remote ischemic preconditioning versus sham

1.5 : DEPENDENT VARIABLES

Running economy, oxygen consumption

Respiratory exchange ratio, submaximal heart rate, minute ventilation

1.6 : ASSUMPTIONS

1. All participants completed medical history questionnaire accurately.

2. All participants followed the protocols as guided.

3. Equipment was working properly.

1.7 : DELIMITATIONS

1. Healthy, recreationally active adult men and women from Brookings, Count

2: LITERATURE REVIEW

2.1 : OVERVIEW

Myocardial I/R injury occurs when cardiac myocytes and coronary endothelial cells are exposed to ischemia that is followed by reestablishment of blood flow (Kloner & Jennings, 2001). The myocytes undergo reversible damage without any permanent damage or cell death if the blood supply is restored within 15 minutes. If the duration and severity of ischemia is of a longer duration, it causes greater reperfusion pathologies which can then lead to reperfusion injury (Kloner & Jennings, 2001; Kloner & Rezkalla, 2006). Reperfusion injury involves a group of local and systemic inflammatory responses that can result in widespread vascular endothelial dysfunction and cellular manifestation characterized by myocardial stunning, endothelial injury and irreversible cell damage and necrosis (Verma et al., 2002). Cellular damage following I/R injury can be alleviated by IPC, a protective phenomenon that has undergone extensive scientific study aimed at reducing myocardial dysfunction following coronary revascularization and myocardial infarction. Recently, while it is clear that reperfusion injury affects all organs and tissues exposed to sustained ischemia, studies have shown that I/R injury also diminishes endothelial function in systemic arteries that supply blood to skeletal muscle. Endothelial dysfunction caused by I/R injury can also be prevented by IPC and studies have provided evidence to indicate that IPC can improve exercise performance by enhancing maximal oxygen consumption, lactate metabolism, and perhaps exercise economy (Bailey et al., 2012; De Groot et al., 2010). Accordingly, the following literature review will discuss the cellular mechanisms that contribute to I/R injury and the factors by which remote IPC protects against it. In addition, a review of studies that have shown favorable benefits of

IPC to improve exercise performance will be discussed.

2.2 : ISCHEMIA-REPERFUSION INJURY

Reperfusion injury following ischemia initiates an inflammatory response in the endothelium causing vasoconstriction, platelet and leukocyte activation, increase radical oxygen species and increased exudation (i.e., leakage) of protein-rich plasma out of the vessels (Hausenloy & Yellon, 2008). The inflammatory response can become generalized and effect multiple organs leading to systemic inflammatory response syndrome or the multiple organ dysfunction syndrome (Eltzschig & Collard, 2004). Severe microvascular dysfunction can lead to 'no-reflow' phenomenon where blood flow does not return during reperfusion. Ischemia negatively impacts multiple cellular metabolic pathways and brings about structural changes (Agati, 1999; Granger, 1999). Prolonged ischemia inevitably causes tissue necrosis. In this respect, there is a reduction incellular oxidative phosphorylation that causes depletion in ATP and phosphocreatine levels. ATP depletion results in altered membrane function because the membrane is driven by ATP- dependent ionic pumps [2]. In all cells, particularly the cardiac myocytes and endothelium, I/R leads to increased oxidative stress, calcium imbalances, expression of pro inflammatory cytokines, and endothelial impairments in blood flow and fibrinolytic capacity (Tapuria et al., 2008). These factors will be discussed below in greater detail.

Oxidative Stress

Generation of toxic levels of ROS plays an important role in the pathogenesis of reperfusion injury. Reactive oxygen species such as a) superoxide anion (O_2^-), b) hydroxyl radicals (OH^-), c) hypocholorous acid (HOCl), d) hydrogen peroxide H_2O_2, and

e) peroxynitrite, a derivative of nitric oxide are produced during reperfusion (Kaminski et al., 2002) During reperfusion there is an excessive accumulation of hypoxanthine by xanthine oxidase that leads to the formation of ROS. ROS are also produced from cytochrome oxidase, and cyclooxygenase, and the oxidation of catecholamines. ROS cause lipid peroxidation and injury to cellular membranes (Eltzschig & Collard, 2004).

Cellular Calcium Overload

Imbalance in calcium homeostasis plays an important role in the development of reperfusion injury. Excessive amount of calcium enters the sarcolemma through L-type calcium channels located in cardiac muscle as well as skeletal muscles. The sensitivity of myofilaments is also distorted (Gross et al., 1999). There is a reduction in the production of ATP due to unavailability of oxygen during ischemia (Gross et al., 1999). Heart shifts to anaerobic metabolism for energy production which leads to increased lactate concentration resulting in decreased pH of the myocytes. Low levels of ATP also interfere with sodium/potassium ATPase pump (Na/K ATPase) causing greater influx of sodium (Na) inside the myocytes (Verma et al., 2002). Reduction in pH activates the sodium ion hydrogen exchanger to maintain the acidic environment of the cells. Sodium ion hydrogen exchanger maintains the increase in intracellular level of Na by efflux of Na and influx of Ca ion (Verma et al., 2002). To maintain the osmolality, Na is pushed out of the cell in exchange for calcium that leads to Ca^{2+} overload in the cell. The excessive Ca in the myocyte goes into the sarcoplasmic reticulum, leading to overload of calcium in sarcoplasm which is again pushed out of the cell. A vicious cycle of calcium uptake and release results in excessive use of ATP and imbalance in Ca homeostasis (Hoffman, Gilbert, Poston, & Silldorff, 2004). The process leads to permanent damage of these

pumps during ischemia (Hoffman et al., 2004). The build-up of calcium inside the cells lead to hypercontracture of cardiac myocytes, that ultimately results in contraction band necrosis which signifies damage to sarcolemma and cell death during reperfusion. Hypercontracture in one myocyte can affect the neighboring myocyte (Hoffman et al., 2004). The uninjured neighbor myocyte is also exposed to excessive calcium through the gap junctions that can lead to calcium overload in the cell. The hypercontraction occurring in the adjacent myocyte can also cause mechanical disruption in the unexposed myocyte through intercalated disks (Hausenloy & Yellon, 2008). Collectively, calcium overload in cardiac myocytes is thought to be a primary reason contributing to I/R injury.

Inflammation

I/R injury increase the expression of certain enzymes and genes that result in inflammation. In a study done by Zager et al. (2009), it was observed that renal ischemia leads to a variation of histone at proinflammatory genes and upregulation of histone modifying enzymes (Set1, BRG1) expression. The study established an associated increase in cognate mRNA and cytokine levels of these genes, which has a relevance to the mechanism of injury after a period of ischemia (Zager & Johnson, 2009).

Endothelial Dysfunction

Reperfusion leads to severe intravascular inflammation and severe endothelial dysfunction. There is a rise in production of the vasoconstrictor endothelin-1 and increased ROS that results in increased vasoconstriction and reduced blood flow. The endothelial dysfunction also disturbs the balance between coagulation and anti-coagulation factors in the blood. Polymorphonuclear neutrophils are transported to the

intravascular space from the interstitial space during ischemia, and such responses may contribute significantly to tissue damage during subsequent reperfusion (Granger, 1999; Jordan et al., 1999). Moreover, Kharbanda and colleagues (2009) were one of first to show that 20 minutes of forearm ischemia impaired endothelium-mediated vasodilation (Kharbanda et al., 2009). In 14 healthy men, blood flow responses to acetylcholine were blunted by ~50% following endothelial I/R injury. Interestingly, subjecting one forearm to remote IPC protected the arm exposed to sustained ischemia against reperfusion injury. In another study conducted by Bailey et al. (2012), remote IPC prevented the reduction in endothelial-mediated vasodilator function of the brachial artery after vigorous lower limb exercise. Thirteen men performed treadmill running followed by a remote IPC protocol. While acute bouts of strenuous exercise has been shown to blunt vasodilation, this study showed that remote IPC was able to prevent it [3]. Furthermore, an experimental study done Pedersen and colleagues showed that endothelial ischemia-reperfusion injury significantly diminishes tissue-type plasminogen activator (t-PA) release, a well-established marker of vascular endothelial health and function, in health men. Surprisingly, IPC could not prevent the reduction in t-PA release in this study. Twenty-two men underwent local IPC, remote IPC and a sham protocol of the forearm following I/R injury caused by suprasystolic upper arm cuff inflation (~220 mmHg for 15 min). Similar to the t-PA response, the investigators observed impaired vasodilation due to infusion of substance P and acetylcholine that was not prevented by remote or local IPC (Pedersen et al., 2012).

2.3: CLINICAL FEATURES OF REPERFUSION INJURY

In the clinical arena, I/R injury contributes to post-operative early mortality,

recurrent myocardial infarction, organ failure, and more extensive cell damage. However, while early restoration of blood flow is required to salvage cells and improve patient survival undergoing surgical procedures, such as percutaneous coronary intervention, reperfusion itself accelerates damage, undermining the effectiveness of prompt reperfusion therapy to restore organ function. Indeed, I/R injury was an underlying feature responsible for early death following coronary artery bypass graft surgery (Weman et al. 2000), despite early and successful reperfusion. In addition, it contributes to adverse outcomes in recipients of organ transplants and in patients undergoing organ resection (i.e., surgical removal of segments of organs), and contributes to organ rejection following successful transplantation (Huang et al. 2009). The clinical features of these adverse changes following I/R injury are characterized by myocardial stunning, reperfusion arrhythmias, and multi-organ dysfunction syndrome.

Myocardial stunning

The term myocardial stunning was first described by Heyndrickx et al. in 1975. It is defined as "prolonged post-ischemic dysfunction of viable tissue salvaged by reperfusion" (Kloner & Jennings, 2001). During myocardial stunning the myocardial cells undergo prolonged I/R which is reversible with time. Typically, myocardial stunning caused by I/R injury leads to impaired ventricular contraction that can last for 1-3 days after an ischemic event (Bolli & Marbán, 1999). The mechanism of myocardial dysfunction includes reduction in ATP synthesis, vascular spasm, intracellular calcium reuptake and increase in ROS (Eltzschig & Collard, 2004). In fact, such brief periods of ischemia are encountered in the clinical situations of angina, coronary vasospasm, and balloon angioplasty, and mostly are not associated with coupled myocyte cell death.

Myocardial stunning is one of the causative factor of cardiomyopathy that in time can lead to cardiac failure (Verma et al., 2002).

Reperfusion arrhythmias

Arrhythmias, ventricular tachycardia, atrial fibrillation and accelerated idioventricular rhythms are common in patients undergoing cardiac surgeries such as coronary artery bypass graft, coronary angioplasty and open heart surgeries involving valvular replacement. Arrhythmias frequently occur in cardiac patients following reperfusion. Reperfusion arrhythmias, which represent the first stage of myocardial I/R injury following 1-5 minutes of sustained ischemia, occur due to abrupt changes in the ionic concentrations. Increasing the blood flow in gradually, in stages reduces the incidence of such arrhythmias and reducing the risk of postoperative mortality (Eltzschig & Collard, 2004).

Multi-organ dysfunction syndrome

Multiple-organ dysfunction syndromes (MODs) are the extreme outcome of I/R injuries. MODs occurs due to the failure of coagulation and immune system leading to clotting, disseminated intravascular coagulation and compromised immune system. Respiratory failure, which is often followed by acute respiratory insufficiency, ensues within 27-72 hours of initial ischemic event (Kaminski et al., 2002). Liver, kidney, gastrointestinal, myocardial and central nervous system deficits follow respiratory failure**.** MODs account for up to 30–40% of intensive care unit mortality (Granger, 1999).

In summary, I/R injury can prove fatal, if goes unchecked. In clinical settings anti- inflammatory drug therapies are used in postoperative conditions to control

exaggerated inflammatory response. However, a promising strategy to prevent or reduce the adverse clinical consequences of I/R injury is to precondition the cells to become more tolerant against a sustained ischemic bout. The following section will detail the mechanisms of preconditioning and its capacity to reduce injury during clinical situations and improve exercise performance.

2.3 : ISCHEMIC PRECONDITIONING

In an early experiment, Murry et al. (1986) showed a reduction in myocardial infarct size in canine hearts after the application of a preconditioning stimulus via occlusion of the circumflex coronary artery (Murry et al. 1986). As a result of this groundbreaking study, the concept of ischemic preconditioning was introduced by these authors in 1986. In this experiment, the circumflex coronary artery was exposed to four 5 min occlusions, each separated by 5 min of reperfusion, which was then followed by continuous ischemia for 40 minutes. They observed a 25% reduction in the myocardial infarct size with the IPC stimulus (Murry et al., 1986). This study laid the groundwork for many subsequent studies showing the protective benefits of preconditioning a tissue locally and remotely.

IPC is an intervention that aims at improving ischemic tolerance of the tissues prior to the actual ischemic insult. Small, intermittent bouts of ischemia and reperfusion provide the target organ a level of resistance against I/R injury. IPC can be applied directly or indirectly to the target organ. The direct (also known as local) form of IPC is not widely practiced clinically because the target organ has to be exposed to the preconditioning stimulus through surgical intervention or through the occlusion of major blood vessels which can cause trauma to the vessels and prove detrimental. On the other hand, remote

IPC was first demonstrated by Pryklenk et al. in 1993 in which they showed that small episodes of ischemia in one vascular bed protected a different area of the myocardium in the canine to sustained coronary artery occlusion. These investigators suggested that preconditioning was mediated by some unknown factors that were released and transported from the preconditioned area to the tissue experiencing I/R injury (Przyklenk et al., 1993). An experiment done by McClanahan et al. also in 1993, showed a reduction in myocardial infarct size in the rabbit when a preconditioning stimulus was applied to the kidneys. (McClanahan et al. 1993). In this manner, remote IPC was used for inducing protection of a distant organ through a peripheral limb or internal organ. In another studydone by Birnbaum et al. (1997), cardioprotection against I/R injury was triggered via lower limb skeletal muscle IPC. Indeed, they showed that occluding blood flow in the gastrocnemius muscle resulted in a reduction of myocardial infarct size by 65% in rabbit heart (Birnbaum et al. 1997). Similarly, Oxman et al. (1997) demonstrated that application of remote IPC to the hind limb of rats using a tourniquet prevented reperfusion arrhythmias (Oxman et al., 1997).

The protection through IPC occurs in two phases. An early window and a late window of protection. The early window of protection wanes after a few hours but it is the most potent against IR injury. The late window of protection then recurs after 24-48 hours and can persist up to 72- 96 hour (Kharbanda et al., 2009). The mechanism of action in early and late window of protection share common features but there are inherent differences. Generally, IPC acts through three basic biological processes; application of ischemic stimulus, the communication of the IPC stimulus via neuronal pathways and blood borne humoral pathways and then induction of the protective effect on the vital

organs such as heart (Baxter & Ferdinandy, 2001), The proposed mechanism is thought to involve the release of adenosine, bradykinin and opioids that bind to target cells exposed to IR injury. The exact mechanism by which the remote IPC stimulus is transmitted to the target organs however not clear, as transmission can be through multiple pathways and requires a combination of humoral, neuronal or systemic mechanism (Przyklenk et al., 1993). Nevertheless, remote IPC has been researched for its potential to reduce the harmful effects of reperfusion injury and protection against myocardial infarction, particularly in the clinical arena. It has been seen that remote IPC stimulus improves cutaneous circulation remarkably (Shimizu et al. 2007). The study done by Kharbanda et al. (2009) demonstrated a normal ventricular function and reduction in myocardial infarct size after prolonged ischemia preceded by remote IPC in animal models. The same study also showed that remote IPC prevented reperfusion induced endothelialdamage (Kharbanda et al., 2009). The application of an IPC preconditioning stimulus also causes an improvement in the blood flow to the heart and improves left ventricular function (Zhou et al., 2007). Many studies have found profound benefit of remote IPC for improving clinical outcomes followed by a cardiac surgery. For example, Hausenloy et al. (2007) found that remote IPC mediated by limb ischemia lowered the concentration of cardiac troponin-T release after surgery. Elevated cardiac troponin-T levels during surgery are believed to be associated with poor health consequences after the surgery (Luca et al., 2013). A long- term follow up study done by Davies et al. (2013), which was designed to determine the long-term benefits of RIPC after percutaneous coronary intervention, demonstrated that remote IPC is associated with reduced levels of troponin-I 6 months after the surgery. Reduction in levels of troponin I

were associated with reduced mortality due to cerebral and cardiac causes in the 6 year

follow-up (Davies et al., 2013). Despite these clear clinical benefits of remote IPC to

reduce cellular injury, it has not been translated into widespread clinical practice. The

precise mechanisms to explain the cellular protection afforded by IPC, particularly how

remote IPC induces protection of a distant organ have been extensively studied. Many

studies were done to understand how the IPC stimulus travels from the effector to the

target organ. The following section will exclusively focus on the mechanisms thought to

be responsible for the favorable effects of remote IPC to protect against I/R injury.

2.4 : MECHANISMS TO EXPLAIN REMOTE IPC

Lim and colleagues (2010) suggested possible involvement of neuronal and

humoral pathways to conduct the remote IPC stimulus. To test this notion, remote IPC

was applied by 3X5 min left femoral artery occlusion each followed by 5 min reperfusion

before prolonged myocardial ischemia in absence and presence of femoral vein occlusion

(humoral pathway) and femoral nerve resection and/or sciatic nerve resection (neural

pathway) in C57BL/6 mice. Remote IPC protected the heart from myocardial damage

during sustained ischemia-reperfusion injury. Interestingly, the study concluded

contribution from both humoral and neuronal pathways in translation of remote IPC

stimulus because much of the protection was abolished when the femoral vein was

occluded and the femoral/sciatic nerve were resected. This study suggests complex and

redundant mechanisms that involve the release of substances from the preconditioned

tissue that are transported to the target organ to induce protection. It also shows that some

of the signal for protection is mediated by the nervous system ([4].

With respect to potential humoral factors, it is thought that this mechanism is responsible for the initial phase of protection because these factors appear to be released immediately into the blood stream following preconditioning. This was shown in a study by Dickson et al. (1999) in which they obtained blood from a preconditioned rabbits that was transfused into unconditioned rabbits that were subjected to myocardial I/R injury. Rabbits that received preconditioned blood demonstrated a reduction in myocardial infarct size by 77%. The proposed mechanism of action suggested by the study was through opioids. (Dickson et al., 1999). Another study by Dickson and colleagues (1999) in which coronary effluent of preconditioned rabbit's hearts was transferred to rabbits that were subjected to myocardial I/R injury. Transfer of the coronary effluent significantly reduced myocardial infarction size compared to rabbits that did not receive the effluent, suggesting a humoral mechanism (Dickson et al., 1999). A study done by Weinbrenner et al. (2002) demonstrated cardioprotection through infrarenal occlusion of aorta in rats. Their findings point to a protein kinase-dependent humoral mechanisms contributing to remote IPC (Weinbrenner et al. 2002). Several other mechanisms that are thought to be responsible in the translation of remote IPC stimulus from the effector organ to the target organ have also been studied. A summary of some of these factors follows.

Opioids. Opioid receptors are present in the neuromuscular junction of heart, skeletal muscles and intestinal tissues (Tapuria et al., 2008). Three identified subgroups of opioid receptors - mu, kappa and delta - are known to play a role in the conduction of preconditioning. All these receptors were identified in the myocardium of rat and participate in the conduction of preconditioning stimulus. Administration of Nalaxone,

which is a nonspecific opioid receptor antagonist, abolishes the cardioprotection induced by remote IPC of mesenteric artery occlusion, thus infers the involvement of opioid receptors in for inducing cardioprotection (Zhang et al., 2006). Preconditioning induced by opioids works by reducing depletion of ATP, reducing the accumulation of lactate, and reducing the recruitment and infiltration of neutrophils and myeloperoxidase activity in preconditioned skeletal muscles. The attenuation in lactate accumulation during early reperfusion is triggered by activation of opioid receptors (Tapuria et al., 2008). Opioids act via G-protein coupled receptor activation of multiple kinases and modulation of mitochondrial and sarcolemmal potassium ATP channels serving as final end-effector. Some studies point towards the involvement of delta and kappa-opioid receptors in remote IPC (Zhang et al., 2006). Weinbrenner et al. (2004) reported that cardioprotection may act via delat-1-opioid receptors (Weinbrenner et al. 2004). In the study done by Zhang et al. (2006), opioid receptor agonist U-50,488H was introduced in blood of mice which mimicked the effect of RIPC induced by femoral occlusion. The cardioprotective effect was absent when opioid receptor antagonist binaltrophimine was introduced (Zhang et al., 2006).

Nitric oxide: Nitric oxide is produced from the metabolism of L-arginine by the enzyme nitric oxide synthase (NOS). There are three isoforms of NOS, endogenous NOS, neuronal NOS and inducible NOS. Inducible NOS is produced only in response to inflammation in the hepatocytes, endothelial cells, Kupffer cells, neutrophils and T-lymphocytes (Abu-Amara et al. 2011). Endogenous NOS has protective effect on ndothelium and is constantly produced in small quantities, which protects the

endothelium. A study done by Abu Amara and colleagues investigated the role of endothelium nitric oxide in inducing remote IPC in mice models. Mice with muted expression of endothelium nitric oxide synthase suffered greater hepatic injury as compared to the wild mice due to reperfusion after ischemia (Abu-Amara et al., 2011). Consequently, mice with an overexpression of endothelium nitric oxide synthase showed resistance to hepatic reperfusion injury (Duranski et al., 2006). To add to it, a study done by Tukono et al. (2008) established that induced NOS acts as a trigger in maintaining remote IPC response rather than a mediator of remote IPC. The study observed deficient preconditioning response due to lack of induced NOS gene in mice. As suggested by some other studies, ROS, cytokines, and NO are generated in venous effluents following intestinal ischemia reperfusion (Tapuria et al., 2008). Collectively, nitric oxide plays a key role underlying many of the protective effects of remote IPC. Nitric oxide protects the microvasculature through vasodilatation, inhibition of neutrophil aggregation, inhibition of stellate cell activation, and by activating cyclic GMP in myocardium and inhibition of cyclic GMP levels and reducing energy demand. It appears that NO also works by activation of PKC, NF-k B and increased transcription of induced NOS in the later phases of remote IPC.

Bradykinin: Bradykinin acts through humoral and neuronal pathways to confer remote IPC to a target organ. Studies by Shoemaker and colleagues showed that intermittent occlusion of the mesenteric artery induced cardioprotection that was mediated by bradykinin (Schoemaker & van Heijningen, 2000). Bradykinin activates efferent nerves which stimulate bradykinin receptor-2 on heart which in turn induces cardioprotection.

Wolfrum et al. (2001) demonstrated that bradykinin activates PKC. The mechanism of action through KATP channels is proposed but not clearly understood.

Adenosine: Adenosine is a purine nucleoside composed of adenine attached to ribose base. It is molecule present in human tissues that can initiate an IPC response as well as can act a as a mediator for remote IPC. Adenosine is produced in muscle cells and endothelial cells. The production of adenosine can increase up to 50 times during ischemic period by the breakdown of ATP (Pell et al., 1998). After the breakdown of ATP, adenosine then diffuses to the interstitial space and reaches the blood stream. The half-life period of adenosine id 0.6-1.5 s and is readily taken up by endothelial cells, RBCs and pericytes which help in metabolizing adenosine. Therefore, it is suggested that adenosine cannot reach the target organ through circulation (Pell et al., 1998). Some studies suggested a neuronal mode of action of adenosine in which it stimulates the afferent nerves locally in the mesenteric bed, which then stimulate adenosine receptors present in the ventricular cardiomyocytes and endothelial cells (Bulbulian et al. 1986). Renal preconditioning induced by renal artery occlusion reduced myocardial infarct size by 65%; however, the preconditioning response was ineffective when nonselective adenosine receptor antagonist 8-SPT or the KATP channel blocker 5-HD was infused before renal preconditioning (Pell et al., 1998). Adenosine may act through adenosine receptors present in the kidneys that release more substances including endothelin, prostaglandin and renal medullary lipids that can provide cardioprotection through K-ATP channels. A study by Pang and colleagues (1997) established a role of adenosine in remote IPC. Adenosine blockade using 8-SPT and free radical scavenger

mercaptopropionyl glycine (MPG) aborted the protection induced by remote IPC. The study also found that on infusion of reserpine the levels of adenosine dropped and beneficial effects of remote IPC were abolished. The study done by Pell et al. (1998) on rabbit models also showed that blockade of adenosine before induction of remote IPC prevented cardioprotection. Taken together, several experimental animal models have provide clear evidence of the favorable effects of adenosine to mediate IPC.

Potassium ATP channels (KATP): KATP channels are present in brain, smooth muscles, skeletal muscles, intestine, kidney and pancreas. KATP channels play an important role in protecting the heart against hypoxic and ischemic stress [5]. KATP channels are present in the sarcolemma as well as in the mitochondria of the cells. Moses et al. demonstrated that administration of nonselective blocker glibenclamide and selective calcium channel blockers 5HD abolished the protective effect of remote IPC, showing the involvement of sarcolemma KATP channels [5]. KATP channels act by reducing depletion of ATP and maintaining phosphocreatine levels and intracellular acidic balance inside the cardiac myocytes after remote IPC [6]. The KATP channels also act by reducing the hydrolysis of ATP and mitochondrial ATPase activity, thus sparing ATP breakdown. Opening of KATP channels preserves calcium balance inside the mitochondria thereby maintaining the electron transfer between mitochondria and ATPase enzymes (Moses et al., 2005; Fairbanks & Brambrink, 2010). Collectively, many studies are underway to determine the mechanisms underlying the cellular protection conferred by IPC against I/R injury. Moreover, several clinical trials are currently underway to translate these protective benefits to reduce myocardial I/R injury by subjecting a patient to short

periods of limb ischemia immediately before a surgical procedure. Recently however, a new area of research has provided preliminary evidence showing that IPC also has important benefits on exercise performance, which may be mediated by mimicking some of the protection induced by exercise preconditioning, a phenomenon that shares some mechanisms as IPC. Indeed, remote IPC has been shown to improve performance in sports such as running, swimming and cycling. It may be that part of the benefits of IPC on exercise performance is mediated by changes in exercise economy.

2.4 : EXERCISE ECONOMY

A person with a better exercise economy will be able to perform at a higher level compared with a person with lower exercise economy even if other factors such as maximal oxygen consumption and lactate threshold are similar. The economy of motion relates to the quantity of oxygen (ml/kg/min) required to move at a given speed or generate a specific amount of power. Exercise economy has been shown to be an important predictor of endurance exercise performance in a number of endurancesports (cycling, swimming, running etc.) and can help to explain differences in performance between individuals. With respect to running economy, differences are determined by various factors such as biomechanical factors joint stability, stride length anthropometrics, kinetics and kinematics and other training factors (Saunders et al. 2004).

2.5 : FACTORS AFFECTING RUNNING ECONOMY

I. **Biomechanical Factors**. Several muscles and joints of the body coordinate for the production of movement at a required energy cost. Altering the mechanics of joint stability

and differences in muscle stiffness can either increase or reduce the energy cost of submaximal exercise. Muscle stiffness has shown to increase power production during bench press. Studies have also shown that strength and power interventions can improve exercise economy and performance by increasing the stiffness of muscles and tendons (Dumke et al., 2010). Kyrolainen et al. (2003) indicated a relationship between myosin heavy chains and titin isoforms in trained runners, which can have an effect on muscle stiffness.

A. *Flexibility*: With respect to running economy, research has established that it can be improved by improving joint stability and reducing flexibility of the hip and calf regions (Craib et al., 1996).To support the literature further, it has been shown that orthotic use improves running economy at low intensity by reducing excessive foot pronation and improving stability of the joints (Burke & Papuga, 2012). Interestingly, it is recognized that sit-and-reach range of motion is negatively associated with running economy (Brown et al., 2011. Indeed, flexibility accounts for 46% of variability in the running economy. A study done by Godges and colleagues (1989) showed that running economy was improved in college athletes by improving the flexibility of the hip flexors and extensors. The authors noted that pelvic balance, improved hip flexibility and myofascial balance are important for neuromuscular balance and contraction to improve running economy. In contrast the study done by Gleim et al. (1990) showed that runners with least flexible muscle at the trunk and pelvic regions had better running economy as the metabolic demand was lowered due to reduced ROM and more stability. In a similar study Creib et al. (1996) reported that lower flexibility in the lower limb especially hip and calf muscles was associated with a better running economy. Improved running economy is dependent

on greater stability of the pelvic muscles and lower need of energy expenditure during the foot strike and more storage of elastic energy in the lower extremities. Other important biomechanical factors affecting running economy per se include stride length, anthropometrics, and kinematics.

      *B. Stride length***:** Trained athletes are known to have longer stride lengths and more efficient exercise economy during running. However, studies have shown that training could not improve stride length and running economy over a period of 7 months in novice athletes. Williams and Cavangh (1987) also showed the impact of other biomechanical factors that affect stride performance with running economy. They concluded that most economical runners possessed lower force peak at heel strike, greater shank angle, smaller maximum plantar flexion angle after toe off and greater forward trunk lean and lower minimum velocity of a point on knee during foot contact.

      C. Anthropometrics: Body weight, height, body fat, and leg length have a critical impact on the running economy. There is a metabolic cost of running associated with the limbs due to their specific angular inertia. A large variation was seen in athletes with similar segmental length and mass. The authors reported an inverse relationship between body mass and maximal oxygen consumption ($r = –0.52$) and between thigh circumference and submaximal oxygen consumption ($r = –0.58$) concluding that the runners with higher mass have a better running economy.

      D. Kinetics and Kinematics: Studies have indicated that running economy is better if the athlete runs at a self-selected stride length compared to a predetermined stride length (Saunders, et al. 2004b). Others concluded that runners tend to choose an optimal stride length and rate with a certain amount of training period for a better running

economy (Williams & Cavanagh, 1987). It has also been proposed that runners tend to adjust their stride length and number according to therate of their perceived exertion for an efficient running economy. Better running economy was also associated with more extended leg during foot strike with longer foot contact time and lower elevation in the vertical peak force. More horizontal heel velocity and greater maximal plantar flexion velocity, lesser arm movements during running was contributor towards running economy (Saunders et al., 2004a).

II. Training

A. Strength training: Improving muscle strength improves running economy by improving muscle coordination, changing motor unit recruitment patterns and conserving elasticity by improving neuromuscular function (Hunter et al., 2011). Strength training can improve the variables associated with running economy such as short contact times and faster forces. Some studies have also demonstrated that resistance training improves endurance in athletes (Bulbulian et al., 1986). A combination of both resistance and endurance training has improved both performance and running economy in athletes. A specific type of strength training called as plyometrics has proven to be beneficial for improving running economy (Turner et al. 2003). Plyometrics cause neural adaptation by increasing the recruitment of motor units without causing muscle fiber hypertrophy. Plyometrics increase tone and stiffness of the muscles and tendons which helps utilize and store elastic energy more efficiently [7]. Paavolainen and colleagues (1999) demonstrated that 9 months of high-intensity strength training can improve running economy by 8% and exercise performance by 3% with no difference in VO2 max with training in moderately trained individuals as a result of improved neuromuscular function.

B. Altitude training: Research indicates that altitude training can improve running economy in sea level runners (Turner et al., 2003). The mechanism can be attributed to increases in hemoglobin levels and oxygen carrying capacity. Weston et al. showed that Kenyan runners who trained at altitude were more resistant to fatigue than Caucasian runners who trained at sea levels despite running at the same treadmill running velocity and similar maximal fitness levels. One mechanism thought to contribute to the improved running performance was reduced lactate production and higher level of oxidative enzymes in Kenyan athletes [8].

While there are a variety of factors and training methods that can improve exercise performance by favorably modifying economy of movement, recent data also points to good potential of IPC to induce exercise benefits. However, to date, no study has directly determined the impact of IPC on exercise economy.

2.5 : ISCHEMIC PRECONDITIONING AND EXERCISE PERFORMANCE

One of the first studies to show benefits of IPC on exercise performance was conducted by De Groot et al. in 2010. They studied the effect of local leg IPC on maximal oxygen uptake in 15 healthy male and female cyclists. The subjects were made to perform incremental maximal exercise test on cycle ergometer on two separate appointments which were a week apart. The exercise protocol, which consisted of an initial cycling power output of 50W sustained for 4 min followed by progression to 100W for 4 min and then 150W for 4 min until exhaustion, was counterbalanced with one day of IPC followed by the maximal exercise test and a control trial (without IPC). Following the IPC trial, the cyclists showed a significant improvement in maximal oxygen uptake by 3%. The maximal oxygen consumption with was 58.4 ml/kg/min with IPC compared with 56.8 ml/kg/min

without it. The maximal power output was also increased (~2%) significantly (from 366W during control to 372W with IPC). Whereas during submaximal testing there was no significant differences in oxygen consumption, respiratory exchange ratio, heart rate or power output. [9]. The authors indicated that a factor contributing to improvement in performance after IPC was due to improvement in the blood flow to the muscles, possibly via KATP channels, adenosine, or nitric oxide, which are thought to be central to the vascular benefits of IPC. This study mainly focused on the effect of early phase of IPC on maximal performance as the participants were made to do the maximal cycle ergometer test 5 min after the application of IPC. However, more studies are needed to investigate the mechanism related to change in vascular adaptations after the application of remote IPC and to ascertain the impact of the late window of protection [9]. Jean et al. in 2011 showed that remote IPC improved maximal exercise performance in highly trained swimmers. Twenty-seven professional swimmers were included in the study, 16 of them completed two submaximal swimming protocols and 18 completed two maximal swim trials consisting of a 100 m swim. Using a randomized crossover study, each subject (within either the submaximal or maximal trials) completed four, 5 min bouts of sublethal ischemia of the upper arm each followed by 4X5 min reperfusions. The remote IPC protocol significantly reduced the time to complete a maximal 100 m swim (an average of 7 sec reduction). There were no differences in submaximal exercise performance with the remote IPC trial. The reason behind no improvement in the submaximal performance is not well understood, however it may be due to different cellular response to remote IPC during maximal and submaximal trials.   Submaximal performance utilizes aerobic pathways for energy production whereas maximal exercise utilizes both aerobic and

anaerobic glycolytic pathways (Jean-St-et al., 2011). In the maximal 100 m trial, the study found that remote IPC group was associated with an increase in the number of strokes without increases in heart rate. It was speculated that a reduced elevation in lactate may underlie the improvement in stroke rate and swimming speed by 0.70-s, which was statistically significant and corresponds to improvement seen with 2 year training program (Jean-St-Michel et al., 2011). Interestingly, samples of dialysate from the human subjects following remote IPC were used to perfuse mouse hearts undergoing myocardial ischemia. The data suggest that remote IPC released a protective factor into the bloodstream of the human subjects, which significantly reduced infarct size in mice exposed to myocardial ischemia. Using a randomized crossover study, Bailey et al. (2012) examined the effects of remote IPC or sham on lactate accumulation and running performance in 13 healthy men. The participants were made to undergo a graded treadmill running test consisting of five, 3 min stages after the application of bilateral upper leg remote IPC (4 cycles of 5 min of ischemia followed by 5 min reperfusion) and a sham trial (4 cycles of 5 min pressure at 20 mmHg followed by 5 min reperfusion). Lactate was assessed after each 3 minute stage. Following a 45 min rest that began after completion of the graded exercise test, subjects performed a 5 km running time trial. Subjects were instructed to choose their own running velocity and to complete the trial as fast as possible. The findings of this showed demonstrated a significant improvement in running performance that was preceded by remote IPC. For example, blood lactate levels during the graded running test were on average 1.1 ± 0.1 mmol/L lower with IPC versus sham (P=0.023). Moreover, subjects significantly reduced their 5-km running time trial duration by 34 sec after IPC versus sham (P=0.027). The authors concluded that IPC

improves running performance by reducing the onset of blood lactate accumulation which allowed for greater work rates. No differences were found in the oxygen consumption between the control and the remote IPC trials during the maximal running test whereas rate of perceived exertion was significantly lower during the IPC trial for the first 1000 km of five kilometer trial time (Bailey et al., 2012). The 5 km run trial was performed 90 min after the application of IPC, suggesting a prolonged window of action of IPC after its application. The mechanism suggested were via improvement in the KATP channels and improved mitochondrial ability to store lactate and oxidation of lactate in the muscle. While most studies discussed thus far have some benefits of IPC on exercise performance, Clevidence and colleagues (2012) report no improvement in cycling performance following remote IPC of the legs in 12 amateur cyclists. Subjects first completed a maximal graded cycle ergometer test beginning at a set power output of 100 W sustained for 5 min. Then the power output was increased by 30 watt/min each min until the participant was exhausted. Several days after the maximal cycling test, subjects completed a submaximal cycling bout that was preceded by either remote IPC or control. During the IPC protocol, cuffs were inflated on the upper part of the thigh at 220 mm of HG for 5 min followed by 5 min of reperfusion. The performance test began with 30%, 50% and 70% of their maximal power output in three stages of 5 min each. Blood samples were taken for analysis of glucose and lactate levels at the beginning and end of each 5 min stage. There were no significant differences between the control and IPC trials with respect to any outcome variable (i.e., oxygen consumption, ventilation, respiratory exchange ratio, heart rate, and blood lactate). The authors concluded that an acute bout of remote IPC did not improve exercise performance   in competitive amateur-level

cyclists (Clevidence et al. 2012). The reasons to explain these negative results are not clear but probably involve the sample population studied. Because the athletes were well experienced cyclists, their maximal fitness capacity, lactate threshold, and cycling economy were already at a very high level and not conducive to further improvement using an acute bout of IPC. Perhaps different results would be demonstrated in novice cyclists.

Taken together, a growing body of evidence suggests a favorable effect of remote IPC to improve exercise performance, particularly maximal performance. It appears that IPC may increase the use of lactate as a substrate which in turn can enhance exercise work rates. Whether IPC affects exercise economy is not clear.

2.6 : LITERATURE REVIEW SUMMARY

Remote IPC prepares the tissue against a sustained ischemic insult. It is a simple noninvasive procedure that can be done at the bed side. Remote IPC acts via complex mechanisms at cellular levels that provide the target organ an ability to tolerate ischemia. Laboratory trials have shown a reduction in myocardial infarct size, arrhythmias and improved post-surgical outcomes after application of remote IPC. The benefits of remote IPC have been translated to enhance exercise performance. The current research is aimed at understanding the effects of remote IPC on running economy.

3: METHODS

The present study was conducted by Ms.Gungeet Kaur, a graduate student in the Department of Health & Nutritional Sciences at South Dakota State University under the mentorship of Dr. Gary Van Guilder. This section outlines the methodology of the present study.

Specific Aim: To determine the effects of remote IPC on running economy. Based on the literature reviewed above, it is hypothesized that remote IPC will improve running economy.

3.1 : EXPERIMENTAL DESIGN

The study was a randomized single-blind crossover study in which subjects completed two submaximal running protocols to assess running economy in the absence and presence of remote bilateral leg IPC.

Timeline: Following IRB approval, enrollment and data collection procedures were initiated in January 2015 and continued through the fall 2015 semester. We expect to complete all data collection procedures were completed by October 2015.

3.2 : SUBJECT RECRUITMENT

Nineteen healthy, recreationally active adult men and women between the ages of 18-40 years were recruited for the study.

Inclusion Criteria

- Injury free and recreationally active as determined by physical activity recall questionnaire.

- Ability to jog on a treadmill at moderate to high intensity for 15 min

- Any race or ethnicity

- Men and women

- Normotensive (blood pressure ≤140/90 mmHg)

- Not taking any medications that could impact IPC

- Apparently healthy and not meeting any of the exclusion criteria listed below

Exclusion Criteria

- Current smoking or current use of tobacco-containing products

- Past smoking or past use of tobacco-containing products

- Recent injury precluding ability to run at moderate-high intensity

- Pregnant or recent pregnancy

- Use of cardiovascular or blood pressure-altering medications or any drug that has been shown to impact IPC

- History or evidence of hepatic, renal, or hematological disease; diabetes or cardiovascular disease

- Recent use of ergogenic supplements

Eligibility Screening: Potential research subjects that have expressed interest in volunteering for the study will be screened for eligibility using an American College of

Sports Medicine medical history form and a standard physical activity readiness questionnaire. Screening was conducted either on the phone, via email, or in person by the graduate student. Individuals that meet eligibility requirements were invited to undergo an informed consent with signature.

3.3 : EXPERIMENTAL PROCEDURES

Following eligibility screening and informed consent procedures, research subjects were randomized to participate in two separate testing sessions (i.e., remote IPC or sham) approximately 1-2 weeks apart and then crossover to complete the second trial (i.e., sham or remote IPC). Prior to each session, subjects were instructed to refrain from maximum effort exercise for the preceding 24 hours and to abstain from ingestion of any supplement or ergogenic aid that may enhance their performance.

Session 1: Body height, weight, waist circumference, percent body fat, resting heart rate and blood pressure were measured during session 1. A description of these specific measurements is below.

Anthropometric measurements

Standing height and body weight were measured with a digital scale (Seca 876 digital scale, Seca Corporation, Hamburg, Germany). Abdominal waist circumference was assessed with a Gulick tape measured at the smallest part of the abdomen, above the umbilicus and below the xiphoid process to the nearest 0.1 cm at the end of normal expiration using standard procedures. Waist measurements were completed twice and averaged. Percent body fat was estimated by air displacement plethysmography (BODPOD, COSMED USA Inc., Illinois).

Cardiorespiratory fitness

Maximum cardiorespiratory fitness was measured by open-circuit spirometry combined

with indirect calorimetry (PARVO medics) in response to an incremental treadmill

running protocol. Following a warm-up, subjects ran at a self-selected brisk pace at 0%

incline for 2 minutes and then every two minutes thereafter, the workload was increased

by raising the incline of the treadmill by 2% until volitional fatigue. Subjects were

equipped with via a mouthpiece and nose clip, and a heart rate monitor to collect expired

oxygen and carbon dioxide. Maximal oxygen uptake was determined if the subject reach

age-predicted maximum heart rate combined with a respiratory exchange ratio >1.1.

Hemodynamic measurements

Non-dominant arm auscultatory resting systolic and diastolic blood pressure was

measured using an appropriately sized stethoscope and sphygmomanometer (Diagnostic

700 Series, American Diagnostic Corp, Hauppauge, NY) following 5 min of seated quiet

rest using standard procedures. Resting blood pressure measurements were performed

twice, separated by 3 minutes, and averaged. Resting heart rate was measured using a 60

second radial pulse count.

Remote IPC trial

Following the baseline measurements described above, subjects completed either the

remote IPC or sham trials and then perform a submaximal running trial. Three, 5 min

cycles of leg ischemia each followed by 5 min of reperfusion was induced to stimulate

bilateral remote IPC of the proximal thighs. To cause ischemia in the legs, a high pressure

cuff was inflated to 220 mmHg (EC20 rapid cuff inflator, DE Hokanson, Inc., Bellevue, WA) while the subjects rests in the supine position. Heart rate and blood pressure was measured during the last minute of each bout of ischemia. The total time to perform the remote IPC trial was approximately 30 minutes (i.e., 15 minutes of intermittent ischemia and 15 min of intermittent reperfusion).

Sham trial

The sham control trial consisted of the same procedure described above for the remote IPC trial except the pressure cuff were inflated to 20 mmHg to avoid ischemia and reperfusion.

Submaximal Running Economy Trial

Within 15 min following the remote IPC or sham trial, subjects were prepared to complete the submaximal running economy trial coupled with indirect calorimetry (TrueOne 2400, PARVO Medics, Sandy, UT). The submaximal running economy trial consisted of 3X5 min predetermined absolute running velocities with progressive increased workload at 0% grade. It was performed on a treadmill in the Health and Human Performance Laboratory. Each subject was set up with a heart rate monitor placed properly on the lower breastbone and watch for the wrist. Air flow and gas calibration of the indirect calorimeter was completed prior to testing. The investigator created a profile in the metabolic testing program using the participant's personal information such as gender, age, height, and weight. Next, the mouth piece, mask, and head gear were placed on subject and adjusted to fit the individual during the trial. A nose piece was provided to prohibit airflow through the nasal cavity during the test. The mask connects to the

metabolic cart via tubing for the measurement of the volume of oxygen consumed and carbon dioxide produced during exercise. Two investigators were present during the testing throughout the study. Following calibration and set up, the investigators explained the protocol to the participant allowing him or her to ask questions before beginning the test. Then a brief 3 minute walking warm-up protocol at 80.5 m/min (3 mph) was completed. Immediately after the warm-up, the treadmill velocity was increased to a predetermined running velocity for 5 min. Thereafter, the treadmill velocity increased to 13.4-26.8 m/min (0.5-1.0 mph) for 5 min and again by 13.4-26.8 m/min for a third 5 min stage. The total time completed for the submaximal running trial was 15 min. The participant then cooled down by walking at a self-selected pace for 3-5 min. Steady-state oxygen consumption, respiratory exchange ratio, ventilatory rate, heart rate, and rating of perceived exertion were recorded every 15 sec during each exercise trial. Running economy was calculated by using an average of the oxygen uptake during the last minute of each 5 min running stage. Running economy was expressed as mlO2/kg/km and mlO2/kg/min at each running velocity for the sham and IPC trials.

Blood Lactate

A small sample of whole blood was obtained via finger prick from the index figure at baseline, during the warm-up phase, at the end of each 5 min running stage, and during the active cool-down phase to determine blood lactate levels (Lactate Plus, Nova Biomedical, UK).

Session 2: At least one week following session 1, subjects' crossover and completed

the alternate trial (either remote IPC or sham). Within 15 min after completion of the IPC trial, subjects completed the identical running protocol as performed in session 1.

3.4 : TIME REQUIREMENT

Because session 1 consisted of baseline measurements, it required more time from the research subjects compared with session 2. Baseline procedures lasted about 1 hour. The remote/sham IPC trial will require 30 minutes. The submaximal running trial required 20 minutes with 15 minutes of preparation time. Collectively, with additional miscellaneous time, session 1 require approximately 145 min (2.4 hours). Session 2 involved the remote/sham IPC followed by the submaximal running trial required 85 min (1.4 hours).

3.5 : STATISTICAL ANALYSES

Data were checked for normality and spread. Measures of central tendency were used to calculate baseline demographic subject data. A two-way repeated measures analysis of variance was used to determine differences in resting heart rate and blood pressure at the end of each 3 minute bilateral IPC and sham phase, and to determine differences in steady-state oxygen consumption, heart rate, minute ventilation, respiratory exchange ratio, and running economy between the submaximal running trials with and without IPC. To determine steady-state data, we averaged the final four data points for oxygen consumption, heart rate, minute ventilation, and respiratory exchange ratio from the 4th to 5th minute of each 5 minute exercise stage. Two-way repeated measures analysis of variance was used to determine differences in blood lactate across the three submaximal running trials with and without IPC. Running economy was determined by dividing steady-state oxygen consumption (ml/kg/min) at

each 5 min stage by the running velocity (m/min) and expressed as $mlO_2/kg/m$ and

$mlO_2/kg/km$. Statistical significance was set at $P<0.05$. Data are represented as

means±SD and analyzed with SPSS version 20 (IBM, Inc.).

4: RESULTS

Nineteen participants volunteered to participate in this study between March 2014 and October 2015. One subject dropped out of the study and therefore the data was collected and analyzed for 18 subjects (12 men, 6 women). Each subject participated in three data collection sessions. On average, each session was at least one week apart. The maximum time between IPC and sham trials during the study was 3 months for one participant. All the participants completed 3 sessions.

Subject characteristics are shown in Table 1. The mean age of participants was 27 years with a mean BMI of 24.6 kg/m^2. To make sure that participants met the inclusion criteria set for the study, each subject was screened before their participation using general health history form and a Physical Activity Readiness Questionnaire (Appendix II). The mean VO$_2$ max was 51.8±5.4 ml/kg/min. Mean systolic blood pressure was 114±14 mmHg and mean diastolic blood pressure was 67±7 mmHg.

Table 4.1. Subject characteristics

Variable	Total Group (N=18)
Sex, men/women	12 / 6
Age, year	27 ± 7
Body mass, kg	74.5 ± 10.8
BMI, kg/m^2	24.6 ± 3.0
Height, m	1.74 ± 0.1
Systolic BP, mmHg	114 ± 14
Diastolic BP, mmHg	67 ± 7
RHR, bpm	60 ± 6
VO$_2$max, ml/kg/min	51.8 ± 5.4

Values are mean ± SD. BMI: body mass index; BP: blood pressure; RHR, resting heart rate; VO$_2$max, maximal oxygen consumption.

Subjective Experience with Bilateral Leg Ischemic Preconditioning

Every participant was asked verbally about their experience during and after the IPC and

sham trials. In general, the rapid increased pressure in the cuffs around the thighs was a

relatively new experience for all of the subjects. They described the feeling initially

during the 1ˢᵗ stage of IPC as slightly uncomfortable feeling of tingling and numbness,

which eventually got better during the 2ⁿᵈ and 3ʳᵈ stages of IPC. One participant

experienced pain during IPC and dropped out of study. Overall, IPC was well tolerated

by the participants and all the trials were successfully completed. All subjects also

completed the submaximal running trials well. There was no incidence of injury during

the study.

Resting Hemodynamic Variables during Sham and Ischemic Preconditioning

Resting Heart rate and blood pressure during the sham and IPC trials are presented in

Table 2 below. Heart rate and blood pressure remained stable during the sham (P=0.79)

and IPC (P=0.09) trials and there were no significant interactions between trials. There

was a significant time interaction (P=0.04) in systolic blood pressure in the sham trial,

such that systolic pressure at the end of third cuff phase was significantly lower compared

with baseline. There were no other differences in blood pressure during the IPC or sham

trials.

Table 4.2. Heart rate and blood pressure changes before and during three, 5 min bouts of bilateral sham and ischemic preconditioning of the legs.

	Heart rate, bpm			
	Baseline	5 min	10 min	15 min
Sham	59±8	59±9	58±7	58±5
Ischemic preconditioning	63±11	60±9	58±9	59±9
	Systolic/Diastolic Blood pressure, mmHg			
	Baseline	5 min	10 min	15 min
Sham	120/64±15/8	115/64±12/7	115/65±11/9	114/64±12/8*
Ischemic preconditioning	120/65±16/7	117/65±13/7	117/67±114/7	118/66±13/8

Values are means±SD. *$P<0.05$ versus stage 1.

Effects of Ischemic Preconditioning on Incremental Treadmill Running

Table 3 demonstrates steady-state heart rate, minute ventilation, and respiratory exchange ratio at the end of each 5 min submaximal running stage between the sham and IPC trials. While heart rate, minute ventilation, and respiratory exchange ratio progressively increased with incremental running, there were no significant interactions between trials. Figure 1 and 2 reports the changes in oxygen uptake and heart rate at each minute of the 15 min incremental running test between the sham and IPC interventions, respectively. There were significant increases (both $P<0.001$) in oxygen uptake and heart rate during each running test. However, there was no time X trial interaction in oxygen uptake or heart rate. Likewise, figure 3 depicts steady-state oxygen consumption for each 5 min running stage. In the sham running trial, oxygen consumption progressively increased

22% (P<0.001) from 34.1 (stage 1) to 41.6 ml/kg/min (stage 3) and 20% (P<0.001) from

34.3 (stage 1) to 41.4 ml/kg/min (stage 3) in the IPC running trial. There was no time X

trial interaction in steady-state oxygen.

Table 4.3. Steady-state heart rate, minute ventilation and respiratory exchange ratio during incremental treadmill running between sham and IPC trials.

	Trial		P value
	Sham	IPC	
Stage 1			
Heart rate (bpm)	152±9	151±11	0.756
V_E (l/min)	66±15	66±15	0.879
RER	0.909±0.040	0.906±0.035	0.766
Stage 2			
Heart rate (bpm)	165±8	164±10	0.629
V_E (l/min)	78±18	79±17	0.363
RER	0.937±0.037	0.935±0.041	0.783
Stage 3			
Heart rate (bpm)	174±6	174±8	0.999
V_E (l/min)	90±19	91±18	0.768
RER	0.968±0.036	0.958±0.036	0.058

Data represent mean±SD. IPC, ischemic preconditioning; V_E, minute ventilation; RER, respiratory exchange ratio.

Figure 4.4. Minute oxygen uptake (ml/kg/min) changes during the 3 staged incremental running tests for the sham and IPC trials.
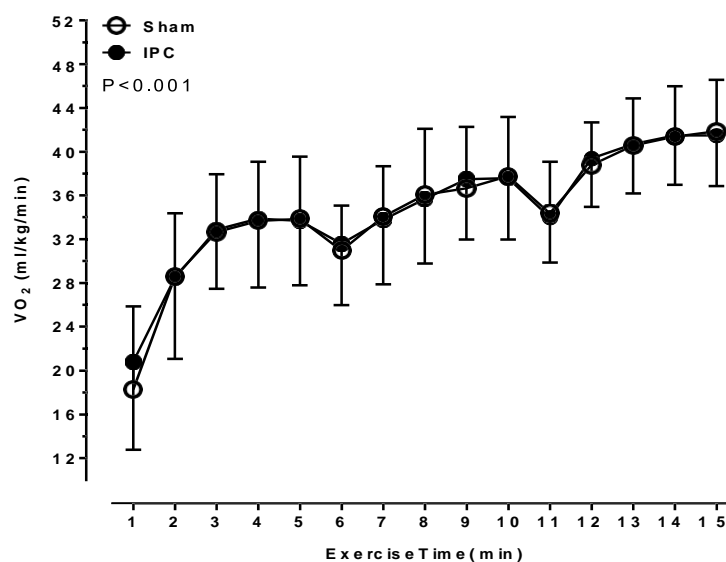
Figure 4.5. Minute heart rate changes (bpm) during the 3 staged incremental running tests for the sham and IPC trials.
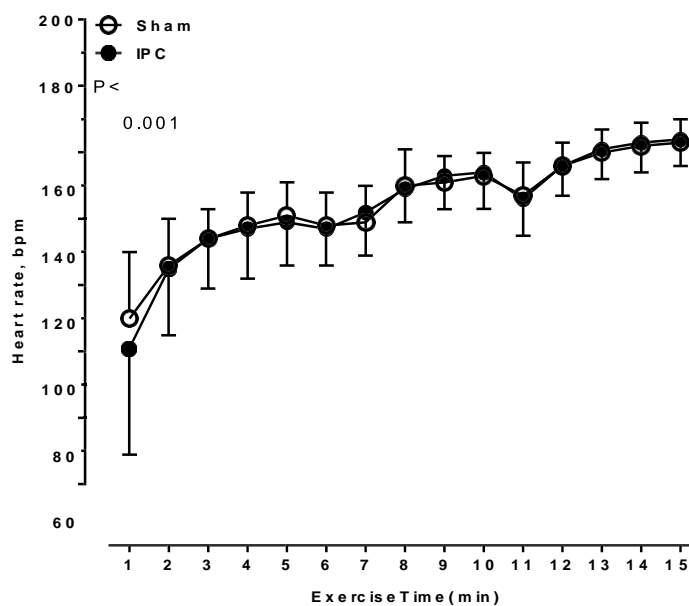
Figure 4.6. Steady-state oxygen uptake at the end of each 5 min running stage between the sham and IPC trials. *P<0.01 versus corresponding stage 1 trial. †P<0.01 versus corresponding trial in stage 1 and stage 2.
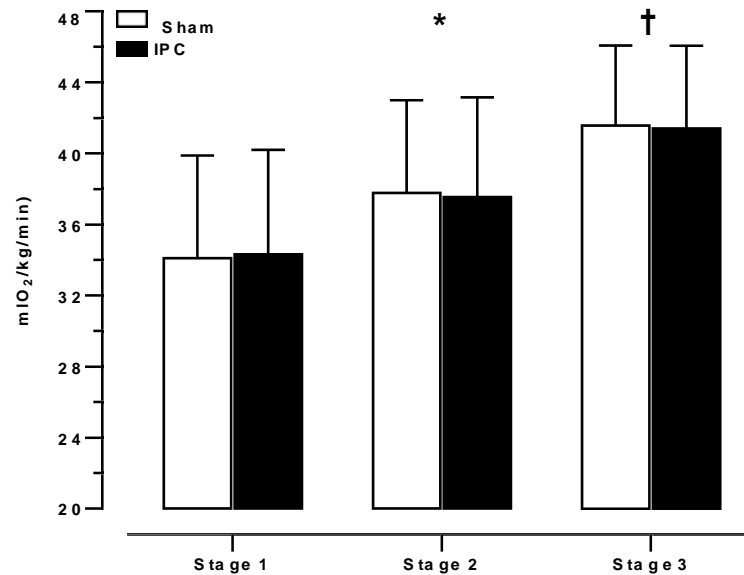
Running Economy

Running economy data between the sham and IPC trials are shown in Figure 4. In the sham running trial, economy remained unchanged (P=0.232) from 201.6 (stage 1) to 204.0 ml/kg/km (stage 3). Similarly, economy did not change in the IPC trial (P=0.129; from 202.8 to 203.1 ml/kg/km). There was no time X trial interaction (P=0.647) in running economy.

Figure 4.7. Running economy at the end of each 5 min incremental running stage in the sham and IPC trials.
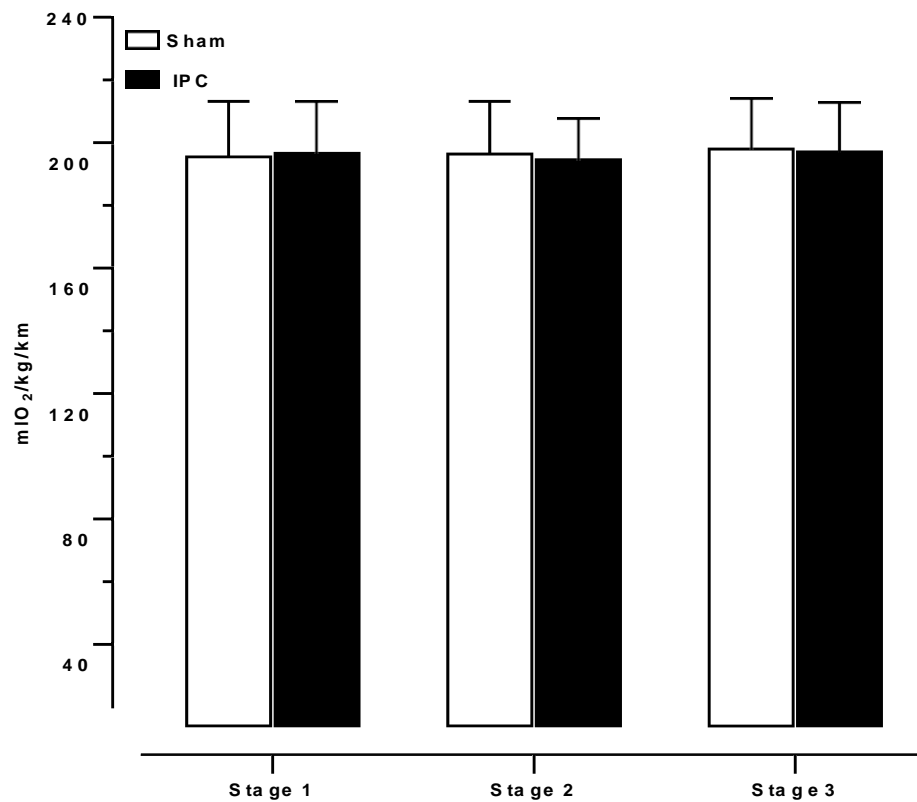
Lactate

Blood lactates are shown in Figure 5. There was a significant increase in lactate during the exercise trials for both sham (P=0.012) and IPC (P=0.001). For example, lactate increased from 3.4±1.6 to 5.3±2.4 mmol/l in the sham trial and from 2.9±1.2 to 5.7±2.2 in the IPC trial. Post hoc analysis demonstrated that lactate at stage 3 was significantly greater compared with stage 1 for both the sham and IPC trials. However, there was no time X trial interaction in blood lactate.

Figure 4.8. Blood lactate at the end of each 5 min incremental running stage in the sham and IPC trials. *P<0.01 versus corresponding stage 1 trial.
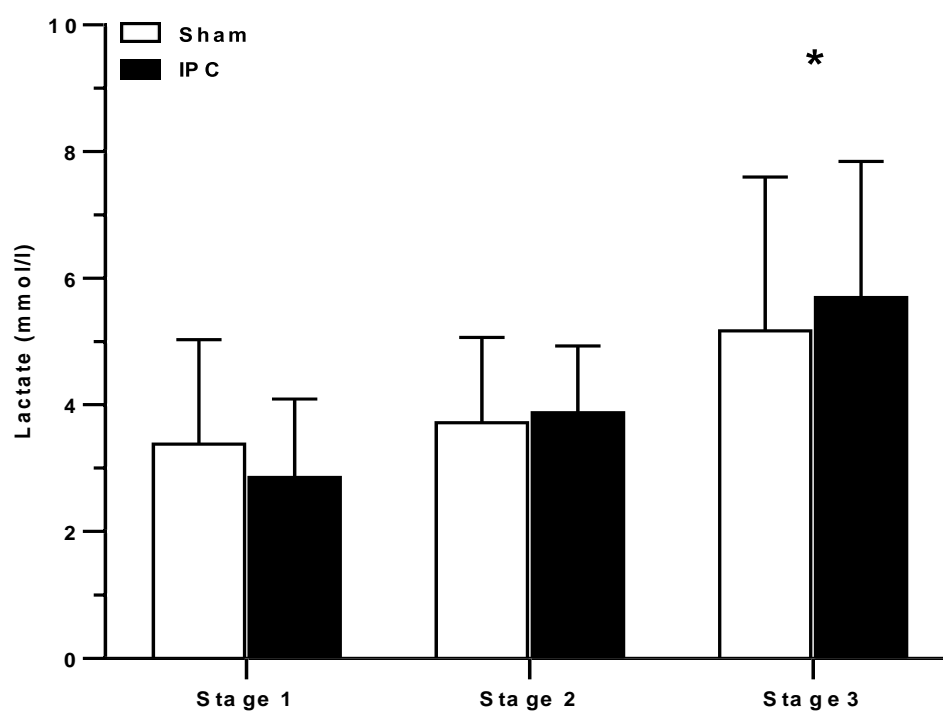
5: DISCUSSION

Summary, Conclusion(s), Limitations, Alternative Hypotheses, Implications for Practice

In the present study, bilateral leg ischemic preconditioning did not affect running economy in recreationally active young healthy adults. These findings are based on similar steady-state oxygen consumption, heart rate, minute ventilation, and respiratory exchange ratio during incremental treadmill running between the sham and preconditioning trials. Thus, the findings of the study do not support our proposed hypothesis and are in contrast to previous studies reporting aerobic exercise performance benefits with ischemic preconditioning of the legs. Collectively, these findings imply that preconditioning may not be the best technique to improve running economy in recreationally active people.

By and large, previous studies have demonstrated that ischemic preconditioning of the legs enhances running, swimming and cycling endurance exercise performance at vigorous exercise intensities but does not influence performance at submaximal exercise intensities. For example, work by Jean et al. on elite swimmers indicated that preconditioning significantly reduced the time to complete a maximal 100 m swim (an average of 7 sec reduction). However, there was no performance benefit in submaximal swimming performance as the authors reported that swimming velocity, heart rate, and the workload at the onset of blood lactate accumulation was similar in the control and conditioning trials [10]. In a different study, De Groot and colleagues reported an improvement in cycling maximal oxygen uptake (3%) and power output with bilateral leg preconditioning during an incremental cycling test but did not demonstrate any improvement in submaximal performance. Indeed, steady-state oxygen consumption,

heart rate, minute ventilation, and respiratory exchange ratio were similar with and without preconditioning in the cyclists [9]. Findings from Clevidence and coworkers (2012) also support the notion that preconditioning does not influence submaximal exercise performance in cyclists. In 12 male cyclists, submaximal heart rate, oxygen uptake, ventilation, respiratory exchange ratio, and blood lactate were not different at 30, 50, and 70% of VO_2max relative exercise intensities between condition and control trials [11]. Collectively, these data suggest that because steady-state oxygen uptake and the workload and/or velocity appear to be similar with and without preconditioning in both swimmers and cyclists, exercise economy would also be expected to remain unchanged. The findings of the present study support this as they provide the first evidence to suggest that economy of running is nearly identical in the absence and presence of ischemic preconditioning. For both trials in the present study, the oxygen cost of running at each velocity was less than 1% different between trials, providing strong support that ischemic precondition of the legs does not improve running economy. In line with the oxygen cost of running data, we also demonstrated that heart rate, minute ventilation, and substrate utilization – as estimated by the respiratory exchange ratio – were unaffected by preconditioning during the incremental running protocol. Moreover, blood lactate concentrations at the end of each steady-state running stage were largely unaffected by preconditioning, arguing for the notion that lactate production and/or metabolism are not contributing to the performance benefits that other groups have reported. It should be noted however, that Bailey et al. did show that the onset of blood lactate accumulation increased during submaximal running at moderate to vigorous exercise velocities and this was associated with faster 5 km running performance. Clearly, more sophisticated

experiments are needed to identify the role that ischemic preconditioning has on lactate metabolism with respect to exercise performance.

It is thought that the factors that modify running economy take time to develop over the course of a runner's career, which is likely to be a primary reason why we did not show improved running economy with a single preconditioning intervention. Running economy depends on various factors including training, exercise environment, and biomechanics [12]. For example, it appears that muscle and joint coordinated movement is particularly important in determining economy of movement. Several muscles and joints of the body coordinate for the production of movement at a required energy cost. Altering the mechanics of joint stability and differences in muscle stiffness can either increase or reduce the energy cost of submaximal exercise. Improving skeletal muscle strength and power has been shown to enhance exercise economy and this performance benefits are due in part to changes in the stiffness of the working muscles and tendons (Dumke et al. 2010). Altitude training has also been shown to improve running economy. Saunders and colleagues (2004) showed that 20 days of live high, train low altitude training lowered the aerobic cost of running in elite runners, however, the improved economy could not be explained by the physiological adaptations that are common to altitude.

It has been suggested that the performance benefits of preconditioning may be due to a placebo effect. Marocolo et al. (2015) recruited 15 amateur swimmers underwent a control, bilateral arm ischemic preconditioning, or sham trial prior to a maximal 100 m swim trial. The study however, made an exception by informing their participants that sham and preconditioning trials would improve performance. The study showed that preconditioning (0.036) improved performance compared to the control trial, but did not

affect performance relative to the sham trial, suggesting that the better swim performance is due to a placebo effect (Marocolo et al., 2015). The potential for a placebo effect has also been shown by this same group of investigators for resistance training (Marocolo et al., 2015).

There are some important limitations of the present study. First, we only investigated the effect of the first window of benefits of preconditioning on running economy. The second window of protection, which begins 12 hours after the conditioning stimulus and last for up to 72 hours, is due in part to gene transcription of new effector proteins that may contribute to exercise performance benefits in athletes. Future studies investigating these potential effects are warranted. Secondly, the present study was limited to the influence of preconditioning on the economy of running and cannot be generalized to economy changes of other modes of aerobic exercise. Thirdly, 17/18 subjects were of Caucasian ethnicity and the findings here cannot be applied to other ethnic groups. Fourth, it is possible that bilateral leg ischemic preconditioning may over condition the skeletal muscle contributing to mild reperfusion injury, which would be expected to interfere with the delivery of blood flow and energy substrates to the working muscle. To address this concern, future studies should consider conditioning one limb instead of two and to remote condition the arm instead of the legs. Interestingly, there are studies that have reported that ischemic conditioning may be detrimental to exercise performance, particularly maximal aerobic exercise. In 15 amateur cyclists, Paixão et al. showed that preconditioning the legs significantly reduced maximal and mean anaerobic power compared to no preconditioning [13]. To date, however, the most studies have reported either improved or no changes in exercise performance with ischemic

precondition, albeit these studies have mostly focused on aerobic exercise. Lastly, we directed each participant to maintain the same level of activity between trials and to avoid running training for the duration of the study. However, we cannot rule out that other lifestyle factors (e.g., exercise habits and nutrition intake) may have influence the findings. Nevertheless, the single-blind, randomized crossover design employed in the present study suggests that, despite these limitations, ischemic preconditioning does not affect submaximal running economy in recreationally active runners.

Conclusion

The results of the study did not support the hypothesis that bilateral leg ischemic preconditioning improves running economy in recreationally active adults. Further studies should be conducted to elucidate the effect of preconditioning modalities on other the energy cost of other sports (i.e., cycling, swimming) and to investigate the potential exercise performance benefits that may be incurred by the second window of protection.

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7: APPENDICES

Appendix-I
Participant Informed Consent Form
Participation in a Research Project
South Dakota State University
Brookings, SD 57007

Department of Health and Nutritional Sciences

Project Director: Dr. Gary P. Van Guilder   Phone No: 605-688-4082

E-mail: gary.vanguilder@sdstate.edu   Date: 12/12/14

Please read (listen to) the following information:

This is an invitation for you, _____, to participate in a research project under the direction of Dr. Gary P. Van Guilder, an Exercise Science Professor.

1. The project is entitled: Effect of Ischemic Preconditioning on Running Economy.

2. The purpose of the project is to determine the effects of remote ischemic preconditioning on running economy. Preconditioning is a process that helps your body become more resistant against physical stress. Some studies have shown that preconditioning may benefit exercise   performance but others have not. Our goal will be to identify if preconditioning improves your running economy, which is an important component of exercise performance.

3. If you consent to participate, you will undergo three research sessions separated at least 1 week. Each research session will require 45 minutes of your time. Each session described below.

Session 1: During the first testing session, we will measure your resting heart rate and blood pressure, body height, weight, waist circumference, and percent body fat. You will also undergo a fitness test on a treadmill.

Measurement of resting heart rate and blood pressure: You will be rest quietly for 5 minutes before we measure your heart rate and blood pressure. Resting heart rate will be measured using a 60 second arterial pulse count. To measure your blood pressure, we will place an inflatable cuff around your upper arm. We will inflate the cuff to a high pressure. Then we will release the pressure slowly while we listen for the sound of your heartbeat. We will repeat this measurement twice separated by 2-3 minutes.

Measurement of body composition: We will measure your height and body weight using digital scale. Abdominal waist circumference will be assessed with a tape measured at the smallest part of the abdomen. Percent body fat and muscle mass will be estimated by air displacement plethysmography. During this procedure, you will be placed in a small enclosed chamber and instructed to sit quietly for 2 minutes. This procedure does not require you do anything except sit quietly.

Remote Ischemic Preconditioning
Preconditioning your muscles has been shown to improve their function. It has also been shown to protect the heart. It can be caused by stopping blood flow to a tissue (e.g. muscle) for very short periods of time (i.e., a few minutes) and then letting blood flow back into the tissue for an equal amount of time. Stopping blood flow in this manner does not damage the tissue. You will undergo preconditioning of your leg muscles. To cause preconditioning in the legs, a pressure cuff will be inflated around both upper thighs to a pressure similar to a blood pressure measurement while you rest on a hospital bed. Three, 5 minutes inflations followed by three, 5 minute deflations will occur. We will measure your heart rate and blood pressure during the last minute of each 5 minute inflation-deflation cycle.

Control trial
The control trial will consist of the same procedure described above for the remote ischemic perconditioning trial except the pressure cuff will be inflated to a low pressure to avoid stopping blood flow to the legs.

Once you have completed either the preconditioning or the control trial, you will perform a 15 minute light to moderate intensity running trial on a treadmill.

Light to Moderate Treadmill Running Trial

Within 15 minutes following the preconditioning or control, you will be prepared to complete the treadmill running protocol in the Health and Human Performance laboratory. We will be measuring the amount of oxygen that you breathe and use to perform the exercise, similar to the procedures described above for your maximum fitness test. To do this, we will equip you with a mouthpiece that will collect your expired air. The air that we collect will be analyzed for oxygen and carbon dioxide. To ensure that we collect your air from your mouth, a nose clip will be placed over your nostrils. You will need to run with the mouthpiece and nose clip for 15 minutes. Once you are set up with the mouthpiece and nose clip, you will walk for 3 minutes to warm up at a walking speed of 3 mph. Immediately after the warm-up, we will increase the treadmill speed to 6 mph. You will maintain this running speed for 5 min. Thereafter, the treadmill speed will increase to 7 mph for 5 min. Lastly, we will increase the treadmill speed to 8 mph for 5 min for a total of 15 minutes of running. Then you will cool down by walking at a self-selected pace for 3-5 min. We will record the amount of oxygen you use, your heart rate, breathing rate, and running economy every minute during each exercise trial.

At end of each 5 minute stage, you will rest for a short period of time (~1 minute) and we will obtain a small drop of blood from your finger using a finger prick to measure the level of lactate. Lactate is a byproduct of your muscle cells that tells us how hard you are exercising. We will perform the lactate measurement 6 times during each exercise test (prior to the start of exercise, at the end of each running stage, and twice during cool down). Your finger will be sterilized with alcohol swabs prior to each lactate measurement. The investigator will wear protective gloves.

Session 3: At least one week following session 2, you will complete the alternate trial (either remote preconditioning or control). Within 15 minutes after completion of that trial, you will complete the identical running protocol as performed in session 2.

1. Participation in this project is voluntary. You have the right to drop out at any time without penalty and you have the right not to participate in any aspect of the study. Your grade for any class will not be affected if you volunteer for this project. You will not get extra credit by volunteering for this study. If you have any questions, you may contact Dr. Van Guilder at the phone number listed at the end of this form.

2. There are minimal risks to you if you volunteer for this project. First, blood pressure measurements involve inflating an upper arm cuff to a high pressure for 20 seconds. You may experience some mild discomfort during this test. Although obtaining a sample of whole blood (one drop, or about 40µl) via a small finger prick is safe and confers minimal physical risk, there is a chance that you will experience mild pain for a few seconds during the procedure. In addition, the finger prick may cause tiny bruising on the skin that should subside in a day. There is a small risk of infection at the finger prick site. With respect to preconditioning your legs, the procedure is quick and poses no more than minimal risk to you. Blood flow to your legs will be stopped for 5 minutes using a high pressure cuff. As a result, you will experience tight squeezing of the upper legs that may be mildly uncomfortable. Although, generally painless, you may experience mild numbness of the legs for 5 minutes. Lastly, when the blood pressure cuff is deflated, you will experience a rush of blood flow through the legs. The skin will redden and feel warm with transient feelings of 'pins and needles' in the skin as blood flow returns to normal. These feelings should subside within 2 minutes.

3. By volunteering for this study you will learn the impact of preconditioning on your ability to run on a treadmill. You will also learn your body composition, maximal cardiorespiratory fitness, and your running economy.

4. You will not be financially compensated by volunteering for the project. There is no financial cost to you for volunteering for this project.

5. Your data is strictly confidential. When the data and analysis are presented, you will not be linked to the data by your name, title or any other identifying item. To keep your identify protected we will assign you a number code. This code will only be available to the research team. The code will link you to the study instead of your name. Your information will be kept confidential in a University computer. The computer is password protected. Copies of data forms will be stored in a locked filing cabinet.

Participant's Signature _____ Date _____

Project Director's Signature _____ Date _____

If you have any questions regarding this study you may contact the Project Director, Dr. Gary Van Guilder, using the contact information presented below. If you have questions regarding your rights as a participant, you can contact the SDSU Research Compliance Coordinator at (605) 688-6975 or SDSU.IRB@sdstate.edu.

<u>Project Director:</u>

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This project has been approved by the SDSU Institutional Review Board, Approval No.: IRB- 1312013-EXP

Appendix-II
Vascular Protection Laboratory
Department of Health and Nutritional Sciences
Eligibility Screening Questionnaire

This Eligibility Screening Questionnaire is necessary to help us understand any potential risks associated with your eligibility in participating in a research study. All information you provide is personal and confidential.

GENERAL INFORMATION

Name	
Address	
City	
State	
Zip code	
Phone (home)	
Phone (work)	
Email	

Age		
Sex		
Hispanic Identity		
Race		

Emergency Contact		Phone	

MEDICAL DIAGNOSES

Do you have or have you ever had any of the following? Please check yes or no.

Con	Yes	No	Condition	Yes	No
Heart attack			Pulmonary disease		
Chest pain			Heart valve problems		
Asthma			Heart murmur		
Anemia			Rheumatic fever		
Cardiovascular surgery			Phlebitis		
Currently pregnant			Emboli (blood clot)		

Emphysema			Coronary artery disease		
Diabetes (type 1 or 2)			Stroke		
Cancer			Peripheral artery disease		

Please list any additional special conditions.

Major Risk Factors	Yes	No	Not sure
Are you a man over the age of 45 or a woman over the age of 55?			
Are you postmenopausal?			
Has your father or brother experienced a heart attack before age 55?			
Has your mother or sister experienced a heart attack before the age of 65?			
Has your doctor ever told you that you might have high blood pressure?			
Is your cholesterol above 200 mg/dl?			
Do you have prediabetes (blood sugar ≥100 mg/dl)?			
Are you physically inactive (less than 30 minutes of physical activity on at least 3 days/week)?			
Do you currently smoke or have you quit smoking in the last 6 months?			
Are you > 20 pounds overweight?			

SIGNS/SYMPTOMS SUGGESTIVE OF CARDIOVASCULAR AND PULMONARY DISEASE	Yes	No
Pain discomfort (or angina equivalent) in the chest, neck, jaw, arms, or other areas that may be due to ischemia (decreased blood flow)		
Shortness of breath at rest or with mild exertion		
Dizziness or fainting at rest or with mild exertion		
Orthopnea/paroxysmal nocturnal dyspnea (labored breathing at night or while sleeping)		
Edema (excessive accumulation of tissue fluid usually in the ankles and lower legs)		
Palpitations or tachycardia (sudden rapid heartbeat)		
Intermittent claudication (lameness due to decreased blood flow)		
Known heart murmur (abnormal heart sound)		
Unusual fatigue		

Cardiovascular diseases include cardiac, peripheral vascular or cerebrovascular disease
Pulmonary diseases include chronic obstructive pulmonary disease, asthma, interstitial lung disease, or cystic fibrosis, Metabolic diseases includes Type 1 or 2 Diabetes mellitus, thyroid disorders, renal or liver .

MEDICATIONS

Please list any medications you are currently taking including prescriptions medications and over the counter medications, dietary supplements, vitamins, minerals, etc.

Medication	Dose and frequency

Please list any dietary supplements you are currently taking including, but not limited to vitamins, minerals, energy drinks, weight loss/weight gain supplements etc.

Dietary supplement	Dose and frequency

Ischemic Preconditioning Running Economy Study
Vascular Protection Laboratory
South Dakota State University
Block Randomization Sheet

Subjet	Trial 1	Trial 2
1.	Sham	IPC
2.	IPC	Sham
3.	IPC	Sham
4.	Sham	IPC
5.	IPC	Sham
6.	Sham	IPC
7.	IPC	Sham
8.	Sham	IPC
9.	IPC	Sham
10.	IPC	Sham
11.	Sham	IPC
12.	Sham	IPC
13.	IPC	Sham
14.	Sham	IPC
15.	Sham	IPC
16.	IPC	Sham
17.	IPC	Sham
18.	Sham	IPC
19.	IPC	Sham
20.	IPC	Sham
21.	IPC	Sham
22.	Sham	IPC
23.	Sham	IPC
24.	Sham	IPC

**Anthropometric and Hemodynamic Data Sheet Vascular
Protection Laboratory
South Dakota State University**

Subject ID:	Tech:	Date:	Trial:

Variable	Value
Age, year	
Height, cm	
Weight, kg	
BMI, kg/m^2	
Obesity status	
Waist circumference, cm	
Hip circumference, cm	
WHR	
WHR status	
Body fat, %	
Resting heart rate, bpm	
Resting blood pressure 1, mmHg	/
Resting blood pressure 2, mmHg	/
Blood pressure classification	

Appendix V
Ischemic Preconditioning Protocol Vascular
Protection Laboratory

Subject ID:	Tech:	Date:	Trial:

Pha se	Time (min)	Ischemia/Reperfusion	Heart Rate, BPM	Blood Press ure.
Bas elin	0	---		
1	1-5 minutes	Ischemia: Cuff inflated	---	--
2	5-10 minutes	Reperfusion: Cuff deflated		/
3	10-15 minutes	Ischemia: Cuff inflated	---	--
4	15-20 minutes	Reperfusion: Cuff deflated		/
5	20-25 minutes	Ischemia: Cuff inflated	---	--
6	25-30 minutes	Reperfusion: Cuff deflated		/

Cuff placement instructions:
Place pressure cuffs on the upper thigh of both legs as high as possible (running economy study only). All other studies place only one cuff on dominant upper arm.

Ischemic preconditioning trial:
Inflate pressure cuffs to 220 mmHg to cause ischemia for 5 minutes, followed by 5 minutes of cuff deflation. Repeat 3 times. Record heart rate and blood pressure during last minute of reperfusion phase.

Sham control trial:
Inflate pressure cuffs to 20 mmHg to avoid ischemia for 5 minutes, followed by 5 minutes of cuff deflation. Repeat 3 times. Record heart rate and blood pressure during last minute of reperfusion phase.

Appendix VI
Ischemic Preconditioning Running Economy
Study Vascular Protection Laboratory

Subject ID:	Tech:	Date:	Trial:

Start Baseline and Warm-up

Level	Protocol Time (min)	Speed, mph (m/min)	HR (bpm)	RPE	Lactate (mmol/l)
Sitting rest	-5-4	---		---	
Warm-up 1	-4-2	3.0		---	
Warm-up 2	-2-0	0.5 less 1st run speed		---	

Start Exercise

1	0-5	5.5 (147)			
2	5-10	6.0 (161)			
3	10-15	6.5 (174)			

S

| Recovery | 15-20 | 2.0-3.0 | | --- | |

At the end of each 5 min stage, instruct the subject to straddle the belt and immediately click 'Pause' on computer. Obtain and record the lactate reading. Select 'Events' and record the lactate reading in the 'RPE' text boxy as, for example 'STG1L3.3, where STG stands for 'stage', '1' stands for Level 1, 'L' stands for lactate and its value. Once the lactate reading has been obtained and recorded, increase treadmill speed to the next level and instruct the subject to resume running and immediately select 'Resume' on the computer. Repeat steps for stages 2-3.

Obtain lactate within 30-45 seconds. Do not change to the treadmill intensity to the next level until you have obtained an accurate lactate reading. Repeat the lactate measurement if necessary.