

# ENHANCING EXERCISE PERFORMANCE THROUGH ISCHAEMIC PRECONDITIONING

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## Authors Declaration

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A handwritten signature in blue ink, appearing to read 'S. Cocking', written in a cursive style.

# Abstract

Ischaemic preconditioning (IPC) is an intervention whereby brief intermittent ischaemic episodes are induced in a limb (usually 4x5-minute arterial occlusion bouts, interspersed with 5-minutes of reperfusion) either at the site of interest (IPC), or at a distance from the site of interest (Remote; RIPC). Although originally linked to cardiology, recently studies have investigated the effects of IPC administered on a limb prior to exercise with some showing improvements in exercise performance. The overarching aim of the present thesis was to investigate how to optimise IPC to further enhance exercise performance.

The optimal protocol of IPC on exercise performance was quantified by manipulating: 1. the number of cycles, 2. amount of muscle tissue, and 3. local vs Remote occlusion, which were applied in a randomized counterbalanced order in study 1 (Chapter 3). IPC dose, location and occlusion area differed prior to a 375 KJ time trial (TT) performance in 12 trained men. The traditional 4x5-min IPC stimulus resulted in the fastest TT time compared to SHAM [17 secs (90% CI: 0, 34 secs);  $P=0.097$ ], but there was no benefit of applying a greater number of cycles [5 secs (-35, 26 secs);  $P=0.49$ ] or employing unilateral IPC [18 secs (-11, 48 secs,  $P=0.29$ ]. Local *versus* Remote cuff placement did not result in changes in TT time [0 secs (-16, 16 secs;  $P>0.9$ ]. Overall, regardless of location, the 4x5-minute dose seemed to provide the most benefit to exercise performance.

The ability of IPC to enhance exercise capacity may be mediated through altering exercise-induced blood flow and/or vascular function. Study 2 (Chapter 4) investigated the blood flow response to exercise, using ultrasound, when exercise was preceded by a control (SHAM) condition, or either local or Remote (R)IPC in eighteen recreationally trained males. Vascular

function tests were also performed before SHAM and (R)IPC and at the end of exercise. IPC resulted in enlarged brachial artery diameter during exercise [0.016 cm (0.003 to 0.03 cm);  $P=0.016$ ] compared to RIPC, but blood flow during exercise was similar between conditions. No changes in post-exercise vascular function were observed between conditions. Therefore, enhanced vasodilation following local (but not Remote) IPC, when performed prior to exercise, does not translate into increased blood flow during exercise nor impact post-exercise vascular function.

IPC could alleviate deleterious muscle damage responses after exercise-induced muscle damage (EIMD; often lasting <72 hours). Study 3 (Chapter 5) investigated whether IPC could negate eccentric exercise-induced reductions in torque production. Eleven recreationally trained males completed 200 repetitions of maximal eccentric contractions when preceded by IPC or SHAM performed in a randomized order, separated by a 9-week washout period. Muscle function tests were performed after IPC/SHAM prior to eccentric exercise and at 1-hour, 24-hours, 48-hours & 72-hours post-EIMD. Venous blood samples were taken at all time points. Greater maximal [15.2 N.m<sup>-1</sup> (6.2 to 24.1);  $P=0.006$ ] and mean [13.3 N.m<sup>-1</sup> (5.3 to 21.3);  $P=0.007$ ] torque production during a fixed angle voluntary maximal voluntary contraction (MVC) task and during a 60 deg·sec<sup>-1</sup> [10.1 (4.9 to 15.3);  $P=0.002$  & 9.8 N.m<sup>-1</sup> (6.1 to 13.5;  $P<0.005$ ] isokinetic task were evident after IPC *versus* SHAM prior to eccentric exercise (EIMD). This was maintained throughout the (72-hour) muscle damage window. Lower cytokine (IL-6 and IL-1ra) were reported after IPC *versus* SHAM ( $P<0.002$ , respectively). IPC resulted in greater overall HSP-27 & 32 levels ( $P<0.01$ ) whilst HSP-72 was lower ( $P=0.001$ ) *versus* SHAM. Therefore, IPC can enhance maximal torque production during isokinetic dynamometry, before and after muscle damaging exercise and induce advantageous extracellular stress responses to EIMD in humans.

Study 4 employed IPC in a practical exercise-priming model, that aimed to maximise repeated sprint ability (RSA). Eleven trained cyclists performed 4 experimental visits in a repeated measures design. The “traditional” 4x5-minute local IPC (IPC) dose was compared to a SHAM condition (20 mmHg). IPC or SHAM were performed on two separate visits, each combined with either passive muscle heating (HEAT) on two visits, or thermoneutral (non-heated) insulation on two visits, prior to an “all out” repeated sprint task (10x6-second sprints with 24-seconds of recovery). There were no meaningful changes in 10x6-second average [12 (-7 to 31) watts;  $P=0.28$ ] or peak [6 (-14 to 26) watts;  $P=0.62$ ] power output following IPC *versus* SHAM. Additionally, no benefit was observed when muscle temperature was elevated in combination with IPC [5 (-14 to 19) watts;  $P=0.67$ ], or separately to IPC [9 (-9 to 28);  $P=0.4$ ] *versus* SHAM. Overall, it appears that IPC, nor (the combination of) muscle heating can positively impact RSA performance in trained cyclists *versus* a SHAM condition.

The findings from this thesis suggest that using a “traditional” dose of 4x5-minute cycles, either on the legs or the arms, promote performance enhancements in aerobic tasks such as cycling TT performance. These potential performance improvements are likely not resultant from increased limb blood flow. IPC can also enhance muscle function following muscle damaging exercise and induce advantageous extracellular stress responses to EIMD. Nevertheless, IPC alone or when combined with local muscle heating likely has no meaningful enhancements in repeated sprint cycling performance. The findings from this thesis may help athletes to establish a better understanding of how IPC can be used prior to exercise.

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# Dedication

I would like to dedicate this work to my parents. Growing up, I don't think I could have asked for better role models in life and I hope this is a step towards making you as proud of me, as I am of you both.

# Candidate Publications

## *Submitted manuscripts based on the work of this thesis*

**Cocking S**, Wilson MG, Nichols D, Cable NT, Green DJ, Thijssen DHJ, Jones H. (2017) Is There an Optimal Ischaemic Preconditioning Dose to Improve Cycling Performance? *Int J Sports Physiol Perform*, 28, 1-25.

**Cocking S**, Cable NT, Wilson MG, Green DJ, Thijssen DHJ, Jones H. (2018) Conduit Artery Diameter During Exercise Is Enhanced After Local, but Not Remote, Ischemic Preconditioning. *Front. Physiol*, 9 (435).

**Cocking S**, Jones H, Cable NT, Thijssen DHJ (2018) Enhancing Sports Performance Through Ischemic Preconditioning: Moderating Factors and Potential Mechanisms, in S.I.S. Rattan & M. Kyriazis (eds), *The Science of Hormesis in Health and Longevity*. Academic Press, London, pp. 213-221.

## *Other publications completed by the candidate during the PhD tenure*

**Cocking S**, Landman T, Benson M, Lord R, Jones H, Gaze D, Thijssen DHJ, George K. The impact of Remote ischemic preconditioning on cardiac biomarker and functional response to endurance exercise. *Scand J Med Sci Sports*, 27(10), 1061-1069.

Racinais S, **Cocking S**, Périard JD. Sports and environmental temperature: From warming-up to heating-up (2017). *Temperature (Austin)*, 4(3), 227-257

## *Oral Communications*

**Cocking, S.** Ischaemic Preconditioning & Exercise Performance: The effect of Dose Manipulation and Cuff Placement. 4th Annual Junior Symposium at ADLQ (JS ADLQ). December 5th, Doha, 2016

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# Chapter 1

## Introduction

Ischaemic preconditioning (IPC) is a method whereby brief intermittent ischaemic episodes are induced in a limb (usually 3 or 4x5-minute arterial occlusion bouts, interspersed with 5-minutes of reperfusion). It was the seminal findings from Murry *et al.* (Murry *et al.*, 1986) that suggested multiple angina episodes preceding myocardial infarction in humans may protect against necrotic cell tissue death, allowing greater salvage of myocardium after ischaemia/reperfusion injury. Subsequently, Przyklenk *et al.* (Przyklenk *et al.*, 1993) demonstrated that IPC could be applied from a distance (Remote (R)IPC) and yet induce a similar magnitude of tissue protection *versus* direct (local) application to a site of injury. These findings expanded the applicability of using IPC to human and animal limbs as a non-invasive tool. To date, this method has been used as a clinical tool in order to enhance a tissues tolerance to ischaemia-induced injury (Barrington *et al.*, 2017). The positive findings in humans (Kharbanda *et al.*, 2001; Kimura *et al.*, 2007) finally lead to the first human exercise study being undertaken (de Groot *et al.*, 2010), resulting in exciting and novel findings.

Over the last eight years, a plethora of research studies (~40 studies) have aimed to determine the impact that IPC (remote and local) can exert in relation to exercise performance. Two systematic reviews (Incognito *et al.*, 2016; Salvador *et al.*, 2016) highlighted the potential for IPC to enhance endurance type events, but also reinforced the need to further investigate the effects of IPC prior to exercise performance. More recently, practitioners and researchers have posed questions such as; “how does IPC exert its effects to enhance exercise capacity?” and “How should this be optimally used to help an athlete?” These questions are critical in determining if, how and when IPC should be used in an elite performance setting, with the sole aim of enhancing athletic performance.

There are several important issues to address before some of these questions can be adequately answered. Firstly, “how should IPC be optimally used to help an athlete?”. Whilst it is suggested

that small improvements in aerobic exercise could be observed following IPC (Salvador *et al.*, 2016), numerous studies have failed to control or standardise how IPC is performed. This has led to wide variations in protocol methodology that differ in: 1) the number of occlusion cycles, 2) tissue occlusion area (unilateral or bilateral IPC) and 3) cuff location (local or Remote cuff placement). The influence of a “dose-dependent” IPC response has therefore been poorly controlled to date. Secondly, a relevant question relates to “how does IPC exert its effects to enhance exercise capacity?”. There are some studies suggesting IPC could mediate its beneficial effects via its impact on metabolic, sympathetic and/or cardiovascular systems (Bailey *et al.*, 2012; Bailey *et al.*, 2012; Horiuchi *et al.*, 2015; Kido *et al.*, 2015). It is possible, based on previous research, that the ability of IPC to enhance exercise capacity may be mediated through altering exercise-induced blood flow and/or vascular function (Bailey *et al.*, 2012; Enko *et al.*, 2011; Jones *et al.*, 2014), but further research is required to understand this concept. These two questions will be directly assessed in the first studies.

It is now well established that tissue-protection is enhanced immediately after IPC (0-4 hours) and again in a delayed (12-72 hours) response (Hausenloy and Yellon, 2010). There has been limited investigation into the effects of delayed preconditioning on exercise performance (Seeger *et al.*, 2016). Nevertheless, this bi-phasic IPC-induced tissue protection against injury, suggest that IPC could protect skeletal muscle injury in response to exercise-induced muscle damage (EIMD), a condition often lasting up to 72 hours after eccentric exercise. Therefore, a third research question is whether IPC can be used to attenuate muscle injury after muscle damaging exercise, offering indirect avenues to promote optimal performance (or to prevent decrements in exercise performance typically experienced after muscle damaging exercise).

Finally, from an athlete/coach perspective, exercise priming strategies, such as IPC, will potentially be employed if found to exert small but meaningful effects on performance. The

commonly implemented recovery time between IPC and commencement of the exercise is often 20-30-minutes (Salvador *et al.*, 2016) which provides an opportunity to try and embed IPC within an optimised exercise priming strategy (Faulkner *et al.*, 2013a; Kilduff *et al.*, 2013a). Therefore, a final aspect worth consideration is “can IPC be combined with other priming strategies to further potentiate the impact of standard priming strategies to exercise performance?”. One commonly utilised priming strategy is increasing muscle temperature, or maintaining muscle temperature after a warm-up with external heating devices/garments (Faulkner *et al.*, 2013a). The mechanisms by which IPC and elevated muscle temperature enhance exercise performance likely differ. Therefore, together they could be additive, if combined prior to an appropriate exercise task.

#### Aims and objectives:

The overarching aim of present thesis is to investigate how to optimise IPC for further enhancing exercise performance. Based on the available research evidence and important considerations of using IPC to enhance exercise performance, the specific study aims are:

1. To examine whether the (i) number IPC cycles (i.e. “dose-cycles”), (ii) the amount of muscle mass occluded (“dose-tissue”), and (iii) the application of IPC to either local or Remote limbs (“Remote”) affect the potency of IPC to improve endurance cycling performance in trained cyclists.
2. To examine the impact of IPC and Remote IPC on exercise-mediated changes in artery diameter and blood flow in healthy young individuals.
3. To investigate whether IPC enhances muscle strength (i.e. torque production) following EIMD in recreationally trained humans.

4. To compare IPC and passive local muscle heat maintenance, or a combination of both interventions, on repeated sprint ability exercise (RSA) performance in trained cyclists.

# Chapter 2

## Review of the Literature

The text originally presented here cannot be made freely available via LJMU E-Theses Collection because of copyright reasons. The text was sourced at <https://www.elsevier.com/books/the-science-of-hormesis-in-health-and-longevity/rattan/978-0-12-814253-0> - (See Appendix 1).

## Chapter 3

### The effect of IPC dose on endurance cycling performance

The text originally presented here cannot be made freely available via LJMU E-Theses Collection because of copyright reasons. The text was sourced at [https://journals.humankinetics.com/doi/abs/10.1123/ijsspp.20170114?rfr\\_dat=cr\\_pub%3Dpubmed&url\\_ver=Z39.88-2003&rfr\\_id=ori%3Arid%3Acrossref.org&journalCode=ijsspp](https://journals.humankinetics.com/doi/abs/10.1123/ijsspp.20170114?rfr_dat=cr_pub%3Dpubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&journalCode=ijsspp) - (See Appendix 2).

# Chapter 4

## The effect of Remote vs Local IPC on blood flow response

The text originally presented here cannot be made freely available via LJMU E-Theses Collection because of copyright reasons. The text was sourced at <https://www.frontiersin.org/articles/10.3389/fphys.2018.00435/full> - (See Appendix 3).



## Chapter 5

# The effect of Ischaemic Preconditioning on Muscle Torque and Systemic Stress Responses to Exercise-Induced Muscle Damage

## 5.1 Introduction

Skeletal muscle damage can be described as the loss of function, usually occurring from either a metabolic- or mechanical-induced stress, resulting in the disruption of muscle structures that are responsible for force production (Newham *et al.*, 1983). Mechanical stress-induced muscle damage is usually a consequence of undertaking either unfamiliar- or eccentric-exercise tasks (Brooks *et al.*, 1995). Acute eccentric work often results in exercise-induced muscle damage (EIMD), typically inducing significant losses in functional capacity and increased pain perception during movements and tasks performed 24-72 hours post-exercise (Howatson and Van Someren, 2008), alongside a greater rate of tension per active fiber (Newham *et al.*, 1983). Accordingly, impaired post-exercise recovery from EIMD may have deleterious impacts on athletic performance during this 24-72 hour EIMD window (Smith, 1992).

EIMD is likely to impact athletes that compete in tournament situations (e.g. team sports), specifically the ability to generate force or power repeatedly (Bishop and Girard, 2013), where time between competitive bouts may be insufficient to ameliorate EIMD responses. This highlights the importance of strategies to prevent or attenuate EIMD. One study, investigated the impact of IPC on eccentric exercise and found bilateral arm IPC prior to a bicep curl eccentric exercise task (3 x 10 repetitions at 80% 1 repetition maximum) reduced CK responses, lowered pain responses and maintained muscle function over a 72-hour period *versus* a SHAM condition (Franz *et al.*, 2017a). Whilst muscle function was assessed via use of radial displacement, no maximal performance tasks were performed. Currently, how IPC influences maximal contraction exercise performance in exercise-induced damaged muscle can only be speculated and this question remains poorly understood. Moreover, limb cuff occlusions applied after muscle damaging exercise (post-exercise intermittent blood flow occlusion bouts) enhanced recovery of muscle damage responses in both isokinetic dynamometry (Page *et al.*,

2017) and countermovement jump performance (Beaven *et al.*, 2012). Taken together, it is possible IPC could enhance the capacity to generate muscle torque following EIMD, but no study to date has aimed to assess performance responses in humans. Additionally, whether IPC enhances recovery kinetics (<72 hours) of maximal torque production following EIMD remains to be established.

During EIMD various cellular processes are activated and expressed to modulate or mitigate deleterious responses to damage, including cytokines such as Interleukin-6 (IL-6) and Interleukin 1 receptor agonist (IL-1ra) (de Oliveira *et al.*, 2011; Nieman *et al.*, 2005). In addition, heat shock proteins (HSPs) are conserved ubiquitous stress proteins that carry out highly varied functions (Noble *et al.*, 2008), with previous studies suggesting that HSP-72 and HSP-32 are involved in the associated resistance to hypoxic stress, ischaemia-reperfusion injury and likely EIMD (Barrington *et al.*, 2017; Stagos *et al.*, 2015; Taylor *et al.*, 2012). HSP-72 targets denatured proteins (Taylor *et al.*, 2012), likely promoting refolding of sub-lethally damaged proteins (Marber *et al.*, 1993), whilst HSP-32 may be important to restore ROS-producing heme-molecule degradation under conditions of low oxygen availability (Gozzelino *et al.*, 2010; Taylor *et al.*, 2012) and EIMD (Stagos *et al.*, 2015). Small HSP's (HSP-27) have previously been shown to not respond to non-damaging endurance exercise in the muscle (Morton *et al.*, 2008), but HSP-27 translocation may help to limit cytoskeletal damage following EIMD (Koh and Escobedo, 2004). Given their involvement in EIMD, the potential effects of IPC on EIMD may be effectively modulated through the systemic cytokine and HSP-response to EIMD in humans.

The primary aim of the current study was to investigate whether IPC could enhance maximal torque production pre- and post- EIMD in humans which is yet to be assessed in the literature. To better understand the potential underlying mechanisms how IPC may influence functional

responses to EIMD, a secondary aim was to evaluate whether IPC mediated favourable systemic cytokine (IL-6 and IL1ra) and HSP stress responses (HSP-72, -32, -27). It was hypothesized that IPC would enhance torque recovery, whilst simultaneously alleviating extracellular stress responses to EIMD via reducing systemic cytokine responses and altering HSP levels before and after EIMD when compared to a SHAM condition.

## 5.2 Methods

### **5.2.1 Location of testing and Ethical Approval**

All experimental protocols and data collection procedures were undertaken in the research testing laboratory of the Athlete Health and Performance laboratory, situated inside Aspetar Sports Medicine Hospital, Doha. All studies included in this thesis were presented to, and approved by both the local chief medical officer scientific committee and the local ethics committee (Anti Doping Laboratory Qatar; ADLQ). Ethical approval number for this study was F2017000229.

### **5.2.2 Inclusion/exclusion criteria**

Participants provided written informed consent. Physical activity readiness questionnaires (PAR-Q) were administered to ensure no participant had any health implications that would prevent participation in the study. All participants were male, between the ages of 18-40 years with no history of operations on the lower limbs and injury free for the previous 6 months. They were regularly training prior to the study to ensure no period of detraining occurred. Participants were excluded if they had any health contraindications, as determined by the PAR-Q or if they were regularly undertaking resistance exercise. All individuals refrained from exercise and alcohol consumption 24 hours, and consumption of caffeine at least 12 hours, respectively, prior to the first laboratory visit.

### **5.2.3 Participants**

Eleven recreationally active males undertaking regular endurance training or team sport competition (mean±SD: age, 33.1±5.3 years; body mass, 78.3±8.3 kg; height 181±7 cm; Resting Blood Pressure, 123±8 / 72±8 mmHg; Resting Heart Rate, 60±8 beats.min<sup>-1</sup>) were recruited. Participants provided written informed consent.

### **5.2.4 Familiarisation Testing**

Resting blood pressure was obtained from participants 5-minutes after arrival (Vital Signs Monitor SP-800, G-Care Electronics Ltd. United Kingdom). Participants were then seated on the isokinetic dynamometer (IsoForce, TUR GmbH, Berlin, Germany) and the dominant leg was securely strapped to the Isokinetic dynamometer lever arm. The lateral femoral condyle was aligned with the dynamometer arm axis of rotation and the knee angle was set to 60 degrees from extension (anatomical zero). The femoral nerve was electrically stimulated during the contraction (voltage 400 V, rectangular pulse of 0.2 ms) via a high-voltage stimulator (Digitimer DS7AH, Digitimer, Hertfordshire, UK) through a cathode placed distally to the inguinal fold and an anode placed laterally to the gluteal fold. The stimulation intensity was adjusted for each individual participant by progressively increasing amperage (starting at 10-miliampere [mA]) by 10-mA increments until a plateau in twitch mechanical response was achieved (peak twitch; PT). The stimulation intensity during Twitch-MVC was calculated by multiplying the minimum electrical intensity required to elicit the PT by 1.5. Once established, participants performed a 5-second MVC. A superimposed doublet stimulation was administered once a plateau in MVC was achieved. Subsequently, a potentiated twitch was evoked 4 s after the MVC, respectively, using doublet stimulation at 100 Hz. Data collected from the force transducer during contractions was subsequently analysed using AcKnowledge v.3.7.2 software (BIOPAC systems inc. Santa Barbara, CA, USA). With the aim of enhancing MVC inter-trial reliability (Shield and Zhou, 2004), at least 2 familiarisation trials were undertaken prior to the first

experimental visit in order to ensure muscular performance was reliable. Data from familiarisation sessions revealed a mean familiarisation MVC value of  $274 \pm 47 \text{ N}\cdot\text{m}^{-1}$ , with a mean intra-individual coefficient of variation (CoV) of  $3.84 \pm 3.86 \%$  for peak torque ( $\text{N}\cdot\text{m}^{-1}$ ). The same equipment, positions, and procedures were used between trials and the isokinetic dynamometer was calibrated to the manufactures guidelines before each test session.

### **5.2.5 Design**

Participants reported to the laboratory and performed either IPC or SHAM in a randomised, counterbalanced order, using a crossover study design (Figure 5.1). For each condition, participants undertook a minimum of two familiarisation sessions, separated by a minimum of 24 hours, to ensure that they were familiar with the muscle function test protocol and maximal force was consistent between visits ( $< 10\%$ ) (Owens *et al.*, 2015). On the first experimental visit, participants lay in a supine position and received either 4x5-minutes of IPC or SHAM, totaling 40-minutes. Participants performed a standardised warm-up before undertaking the baseline (pre-ECC (eccentrics)) muscle function test protocol (consisting of 1 x MVC (maximal voluntary contraction) [superimposed twitch technique; Twitch-MVC], 1 x voluntary MVC [without stimulation; MVC-2], 1 x isokinetic task at  $60 \text{ deg}\cdot\text{sec}^{-1}$  [IKD-60] and 1 x isokinetic task at  $120 \text{ deg}\cdot\text{sec}^{-1}$  [IKD-120]) all of which were performed on the dominant leg. Following this a unilateral leg eccentric-exercise (200 repetitions) protocol commenced on the dominant leg, with the intention of inducing muscle damage (EIMD). The muscle function test battery (MVCs and IKDs) was then repeated 1-hour post-EIMD (post-1), 24-hours post-EIMD (post-24), 48-hours post-EIMD (post-48) and 72-hours post-EIMD (post-72). Venous blood samples were obtained prior to IPC or SHAM, immediately post-IPC/-SHAM, then at the same time points as the muscle function assessments. Participants were instructed to refrain from exercise 24 hours prior to the experimental visit and for the duration of trial (i.e. between baseline and post-72). They then underwent a 9-week washout period to limit the impact of the repeated bout

effect, and were instructed to return to, and maintain their normal physical activity routines, whilst not conducting heavy lower leg resistance training.

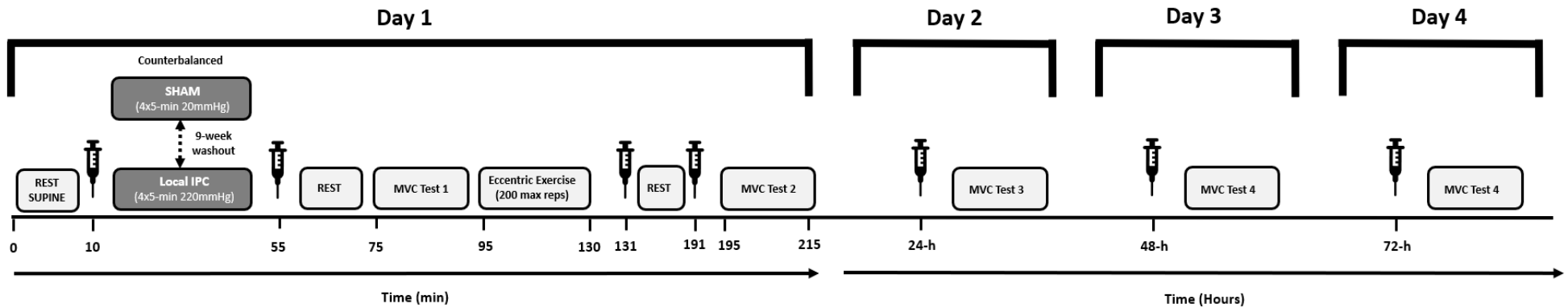


Figure 5. 1. Protocol schematic detailing experimental laboratory visit order of events.

### 5.2.6 IPC protocols

For both the IPC and SHAM trials, a 13.5 cm wide cuff were placed bilaterally on the most proximal portions of the upper thighs. Participants lay in a supine position and cuff inflation pressure was standardised at 220 mmHg for the IPC condition and 20 mmHg in the SHAM condition, respectively. Cuff pressures were attained with the use of an automatic rapid cuff inflator (Hokanson, Washington, USA). Pressure during IPC (220 mmHg) and SHAM (20 mmHg) was held for a duration of 5-minutes, interspersed with 5-minutes of complete deflation (0 mmHg).

### **5.2.7 Muscle Function Test Protocols:**

To quantify torque production in laboratory settings, isokinetic dynamometry and fixed angle maximal voluntary contraction protocols are commonly employed (Owens *et al.*, 2015). Whilst not entirely specific to competition, these tasks can provide mechanistic inference to performance and show moderate correlations with certain performance markers in athletes (Saliba and Hrysomallis, 2001). Participants were seated with the knee angle positioned at 60° from extension (anatomical zero) and the dominant leg was securely strapped to the Isokinetic dynamometer lever arm. Pre-twitch (PT) torque was measured using a singlet stimulation at the intensity determined from familiarization testing. After a standardised warm-up (50% MVC x 10 repetitions, 75% MVC x 3 repetitions, 1 x near maximal (<1 second) repetition), participants waited 1-minute before producing a 5-second MVC effort (Twitch-MVC). To elicit Twitch-MVC, a doublet stimulation at 100 Hz was administered (approx. 3 secs into the contraction) once a plateau in MVC was achieved. A post-contraction potentiated twitch was evoked approx. 3-secs after relaxation, using a doublet stimulation at 100 Hz. Data collected from the force transducer [Pre-Twitch (PT) torque; defined as singlet pre-warm up stimulation, maximal torque (maximal torque); defined as peak voluntary torque attained, percentage maximal involuntary activation (% activation); defined as the % of voluntary activation from doublet stimulation torque value, and post-contraction potentiated stimulation torque (Post-stim); defined as torque attained from the post-contraction doublet stimulation] during contractions were subsequently analysed using AcKnowledge v.3.7.2 software (BIOPAC systems inc. Santa Barbara, CA, USA). Criteria to reject a maximal voluntary contraction was: 1. the force trace exhibited no clear plateau prior to the superimposed stimulation; 2. the superimposed stimulation was delivered when voluntary force was not at or close to its maximum for that contraction; 3. the participant perceived that their effort was submaximal at the time of stimulation (Shield and Zhou, 2004). Following a 3-minute recovery from Twitch-MVC, an additional voluntary MVC



(MVC-2) was performed, without stimulation to elicit maximal voluntary contraction responses. Following Twitch-MVC and MVC-2 tests, two isokinetic tasks were performed; each test separated by 3-minutes of rest. The isokinetic test protocol consisted of four maximal contractions at two different fixed movement velocities. Firstly,  $1.05 \text{ rad}\cdot\text{sec}^{-1}$  ( $60 \text{ deg}\cdot\text{sec}^{-1}$  [IKD-60]) and secondly at  $2.09 \text{ rad}\cdot\text{sec}^{-1}$  ( $120 \text{ deg}\cdot\text{sec}^{-1}$  [IKD-120]). Mean (the average torque value for each contraction) and peak (the maximal torque value during contraction) torque ( $\text{N}\cdot\text{m}^{-1}$ ) was calculated for each repetition, and the varying rate of contractions were separated by a 3-minute recovery period.

### **5.2.8 Eccentric Exercise Protocols:**

Participants were strapped to the isokinetic dynamometer, with the dominant leg attached to the dynamometer arm. They then performed 20 sets of 10 maximal repetitions at a constant speed of  $30 \text{ deg}\cdot\text{sec}^{-1}$ , equating to a total of 200 eccentric contractions (Owens *et al.*, 2015b). Each set was interspersed with a 30-second recovery interval. Between repetitions, the isokinetic dynamometer arm was passively raised at a speed of  $30 \text{ deg}\cdot\text{sec}^{-1}$  prior to the participant commencing the subsequent contraction. Participants were instructed to produce a maximal effort for each repetition performed. Range of motion was standardised between blocks to ensure contraction length did not vary and total work done (kJ) was recorded for analysis.

### **5.2.9 Exercise Discomfort – Delayed Onset of Muscle Soreness (DOMS) ratings:**

A VAS scale (0-10) was used to determine perceived discomfort during sets 1-10 and sets 11-20 of eccentric-exercise (ECC). The same VAS scale was used to monitor the magnitude of delayed onset of muscle soreness (DOMS) ratings for each of the 5 MVC function tests, with 0 being no DOMS, and 10 being worst possible DOMS.

### **5.2.10 Venous Blood Sampling:**

For the assessment of biochemical stress markers, venous blood samples (8 mL) were drawn from an antecubital vein, with use of an elasticated tourniquet (Latex free tourniquet, BD vacutainer™ systems, NJ, USA)). The tourniquet was deliberately applied for <1 minute to avoid localised haemoconcentration to prevent calculation of erroneous serum data (Nikolac *et al.*, 2013). Samples were collected at rest in supine position. Serum was collected using 2 x SST™ 13 x 100 mm (4 mL volume) blood collection tubes (BD Vacutainer®, Becton, Dickson and Company, Oakville, Ontario) containing spray-coated silica and polymer gel for serum separation. Venous samples were taken at seven separate time points for each experimental condition (Prior to IPC or SHAM (Pre-IPC), immediately post-IPC or SHAM, immediately (Post-IPC), immediately post eccentrics (Post-ECC-0), 1-h post eccentrics (Post-1), 24-h post eccentrics (Post-24), 48-h post eccentrics (Post-48) and 72-h post eccentrics (Post-72). Blood samples were centrifuged ~90-minutes after collection in a swinging bucket rotor of the Multifuge® 1S/1S-R for 10-minutes at 3000-rpm at a temperature of 24 degrees Celsius. The resulting serum was then manually pipetted (Eppendorf, Hamburg, Germany) into Eppendorf low bind protein tubes (Eppendorf, Hamburg, Germany). Eppendorf tubes were then stored at -80 degrees Celsius in a laboratory freezer (Thermo Fisher Scientific, (Asheville) LLC, NC, USA).

### **5.2.11 Enzyme-Linked Immunosorbent Assay:**

Heat shock 27kDa protein (HSP-27) (ng/ml), HSP-32kDa (HSP-32) (ng/ml), HSP-72kDa (HSP-72) (ng/ml), interleukin 1 receptor antagonist (IL-1ra) (pg/ml) and interleukin 6 (IL-6) (pg/ml) concentrations were determined using commercial kits (Cloud-Clone Corp., Texas, USA). The limits of sensitivity were  $\leq 0.31$  ng/ml,  $\leq 0.119$  ng/ml,  $\leq 0.6$  ng/ml and  $\leq 3.3$  pg/ml, respectively. The mean intra-assay coefficient of variation (CV) reported for these parameters were <10%. An automatic enzyme linked immunosorbent assay (ELISA) microplate reader (Infinite® 200

PRO NanoQuant, Switzerland) and Magellan Standard software (version 7.1) were used. A Sunrise microplate absorbance reader (Tecan, Grödig, Austria), and Magellan Standard software (version 7.1, Tecan Group Ltd. Männedorf, Switzerland) were used to analyse samples.

### **5.2.12 Statistical Analysis:**

When assessing torque responses to muscle damage the primary outcome variable is torque production ( $\text{N}\cdot\text{m}^{-1}$ ) for each exercise task. Maximal torque ( $\text{N}\cdot\text{m}^{-1}$ ) for MVC superimposed and MVC (without stimulation) and both mean and maximal torque ( $\text{N}\cdot\text{m}^{-1}$ ) for IKD-60 and IKD-120 tasks were analysed using repeated measures general linear modelling ((Condition [2 levels]: IPC vs SHAM) x (Time Point [5 levels]: Pre-ECC, Post-1, Post-24, Post-48, Post-72)). Secondary outcome measures were analysed using repeated measures general linear modelling ((Condition [2 levels]: IPC vs SHAM) x (Time Point [7 levels]: Pre-IPC, Post-IPC, Post-0, Post-1, Post-24, Post-48, Post-72)). The least significant difference (LSD) method was employed for pairwise comparisons (Perneger, 1998). The level of significance (alpha) was set at  $P=0.05$ . Any  $P$  value that was reported as “0.00” in SPSS was reported as “ $P<0.005$ ”.

## **5.3 Results**

### **5.3.1 Eccentric Exercise**

The total work done (kJ) during each eccentric exercise bout was comparable between SHAM and IPC (main effect of condition:  $P=0.22$ ). Rate of perceived exertion and perceived discomfort throughout the IPC and SHAM 20-sets of eccentric exercise were also not different ( $P>0.05$ ; Table 5.1). The DOMS ratings at the various post-exercise time-points increased following eccentric exercise, with peak DOMS score at the post 48-hr time point [3.6 (2.9, 4.2) / 10 VAS score; Table 5.2] when compared with all other time-points ( $P<0.05$ , respectively).

There was no main effect of condition nor condition x time interaction in DOMS ratings ( $P>0.05$ , Table 5.2).

Table 5. 1. Data representing physical and perceptual ratings during eccentric exercise bouts between conditions. RPE derived from Borg (6-20) scale whilst perceived discomfort derived from VAS scale (0-10).

	Eccentric Exercise		P value
	IPC	SHAM	
Total work done (kJ)	40 ± 3.3	37.4 ± 6.3	0.45
RPE (Sets 0-10)	15 ± 2	15 ± 2	0.94
RPE (Sets 11-20)	16 ± 3	17 ± 2	0.68
Discomfort rating (Sets 0-10)	4 ± 1	4 ± 2	0.85
Discomfort rating (sets 11-20)	5 ± 2	5 ± 3	0.68

Table 5. 2. Summary of the Delayed Onset of Muscle Soreness (DOMS) scores prior to each muscle function test. Scores derived from VAS scale (0-10).

	DOMS rating		P value
	IPC	SHAM	
Pre ECC	0 ± 0	0 ± 0	0.69
Post ECC (1h)	0 ± 1	0 ± 1	
Post ECC (24h)	2 ± 2	2 ± 1	
Post ECC (48h)	4 ± 3	3 ± 3	
Post ECC (72h)	3 ± 3	2 ± 2	

### 5.3.2 Twitch-MVC

There was a main effect of time for all variables, with a reduction from pre-ECC to post-damage time points in PT torque ( $P<0.005$ ), maximal torque ( $P<0.005$ ) and post-stim ( $P<0.005$ ; Table 3). No main effect of condition was observed in PT torque, maximal torque, % activation or post-stim ( $P>0.05$ , respectively) (Table 5.3). No effect of time was observed for % activation ( $P=0.28$ ). There was no evidence of condition x time interactions for any Twitch-MVC ( $P>0.05$ , respectively).

Table 5. 3. Twitch-MVC variables for both IPC and SHAM throughout various time-points

	Pre-ECC		Post-1		Post-24		Post-48		Post-72		P value
	IPC	SHAM	IPC	SHAM	IPC	SHAM	IPC	SHAM	IPC	SHAM	
PT (N.m <sup>-1</sup> )	33.8±4.9	33.7±5	23.9±6	24.1±7.6	34.7±5	32.8±6.2	39.5±6.8	34.7±9.5	38.6±7.3	36.8±5.5	0.5
Maximal torque (N.m <sup>-1</sup> )	268.6±39.3	266.8±53.8	214±32.7	215.2±48.6	240.4±50.9	239.1±53.5	242.3±50.5	230.1±54	255.1±64.5	238.5±52.3	0.98
% activation (% of max)	98.3±12.5	94.9±15.2	94.1±27.7	94.2±19.3	92.2±8.8	92.9±17.4	93.8±10.8	94±19.2	96.9±17.8	86.4±17.1	0.4
Post-stim (N.m <sup>-1</sup> )	100.9±19.9	88.5±20.1	78.5±18.3	74.3±15.4	88±14.1	90±10.3	92.3±18.1	91.4±15.9	100.2±21.5	93.3±16.6	0.23

### 5.3.3 MVC-2 (without stimulation)

There was a main effect of time for both maximal and mean torque ( $P<0.005$ ), with both reducing by  $50.3\pm 8$  and  $47\pm 11$  N.m<sup>-1</sup> at post-1 and then gradually returned to pre-ECC levels at each time point (post-24 to post-72). IPC was associated with a significantly higher maximal torque of 15.2 N.m<sup>-1</sup> (6.2, 24.1;  $P=0.006$ ) and mean torque of 13.3 N.m<sup>-1</sup> (5.3, 21.3;  $P=0.007$ ) compared to SHAM (Figure 5.2). There was no evidence of a condition x time interaction ( $P>0.05$ ).

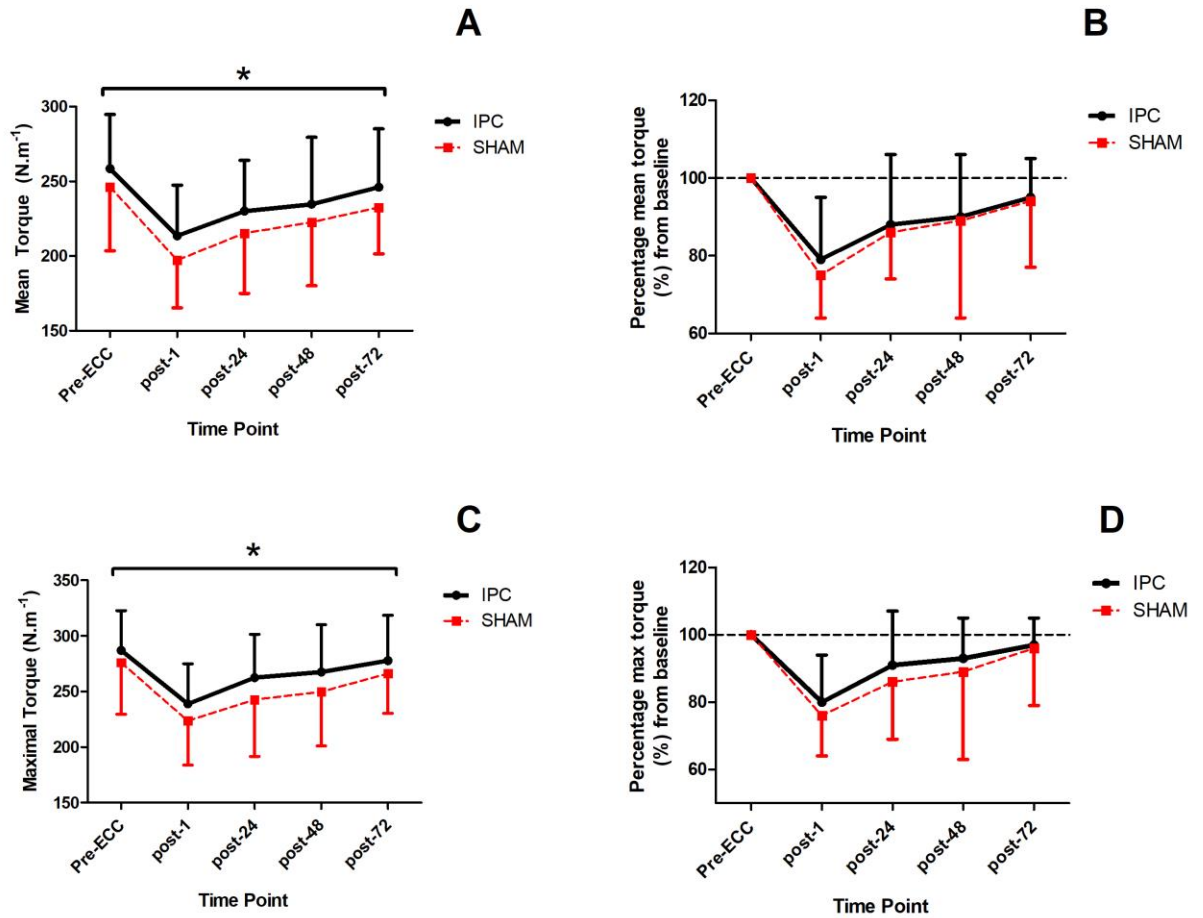


Figure 5. 2. Mean (A) and maximal (C) torque displayed for MVC-2 (without stimulation) responses to EIMD after IPC or SHAM treatments. Absolute Torque production values displayed on the left, with relative changes compared to pre-ECC in mean (B) and maximal (D) Torque values displayed on the right. \* represents statistically significant differences between condition

### 5.3.4 Isokinetic tasks (60 & 120 deg·sec<sup>-1</sup>)

Maximal and mean torque decreased by similar magnitudes at post-1 for both IKD-60 (37.6 & 26.8 N.m<sup>-1</sup>, respectively) and IKD-120 (35.3 & 27.6 N.m<sup>-1</sup>). Maximal and mean torque for both tasks gradually returned to pre-ECC levels at each subsequent time point (post-24 to post-72, main effect of time  $P < 0.05$ , respectively). During IKD-60, IPC was associated with a higher maximal torque of 10.1 N.m<sup>-1</sup> (4.9, 15.3;  $P = 0.002$ ) and mean torque of 9.8 N.m<sup>-1</sup> (6.1, 13.5;

$P < 0.005$ ) compared to SHAM (Figure 5.3). No significant improvements were reported for maximal or mean torque in the IKD-120 task ( $P > 0.05$ ). There was no evidence of a condition x time interaction ( $P > 0.05$ ).

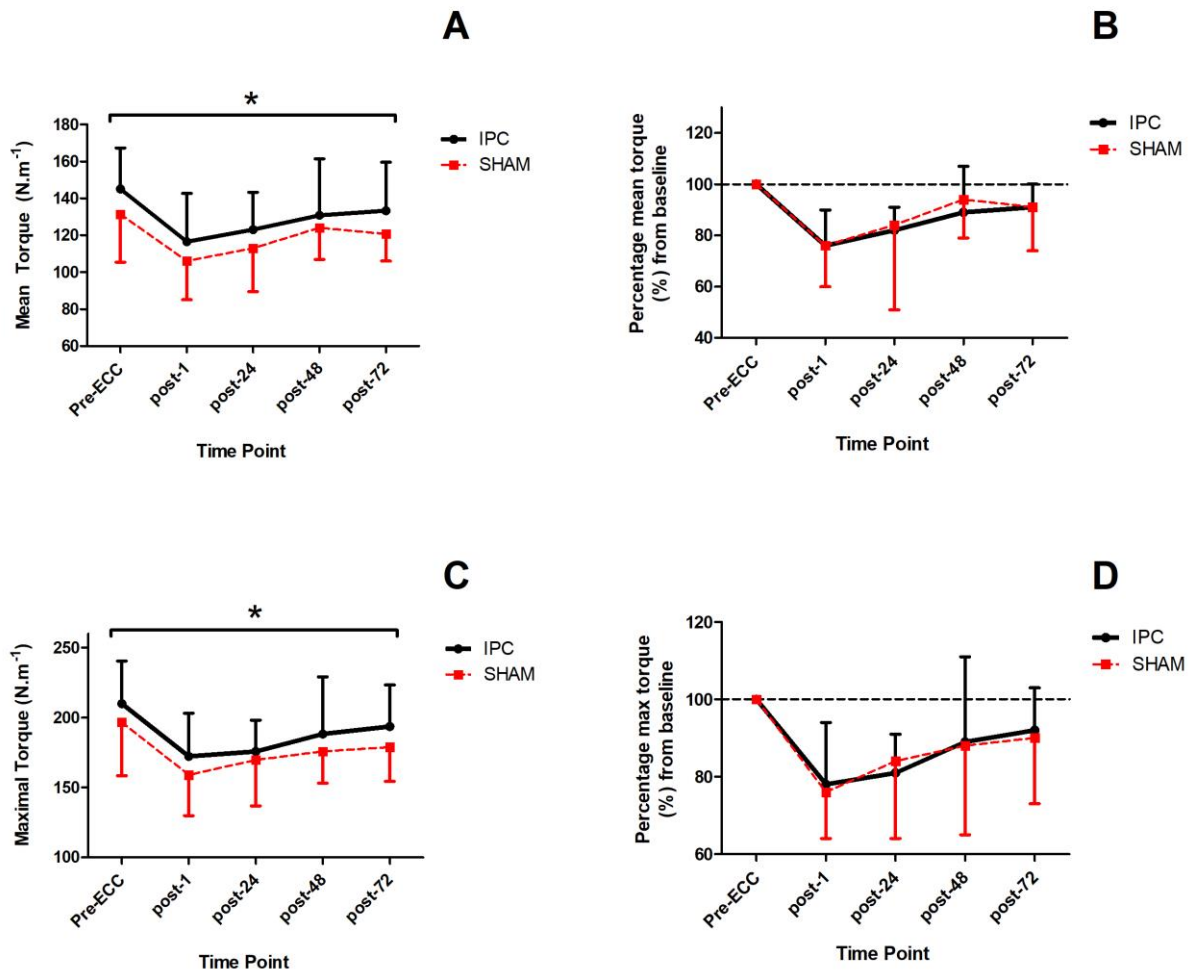


Figure 5. 3. Mean (A) and maximal (C) torque displayed for IKD-60 responses to EIMD after IPC or SHAM treatments. Absolute Torque production values displayed on the left, with relative changes compared to pre-ECC in mean (B) and maximal (D) Torque values displayed on the right. \* represents statistically significant differences between condition

### 5.3.5 Cytokine Responses (pg/mL)

A main effect of time was observed for serum IL-1ra ( $P < 0.005$ ) but not serum IL-6 ( $P = 0.37$ ; Figure 5.4). Serum IL-1ra [-7.9 (-12.3, -3.4) pg/mL;  $P = 0.001$ ] and IL-6 [-0.39 (-0.63, -0.15)

pg/mL;  $P=0.002$ ] were lower following IPC *versus* SHAM. There was no condition x time interaction for IL-1ra or IL-6 ( $P>0.05$ , respectively; Figure 5.4).

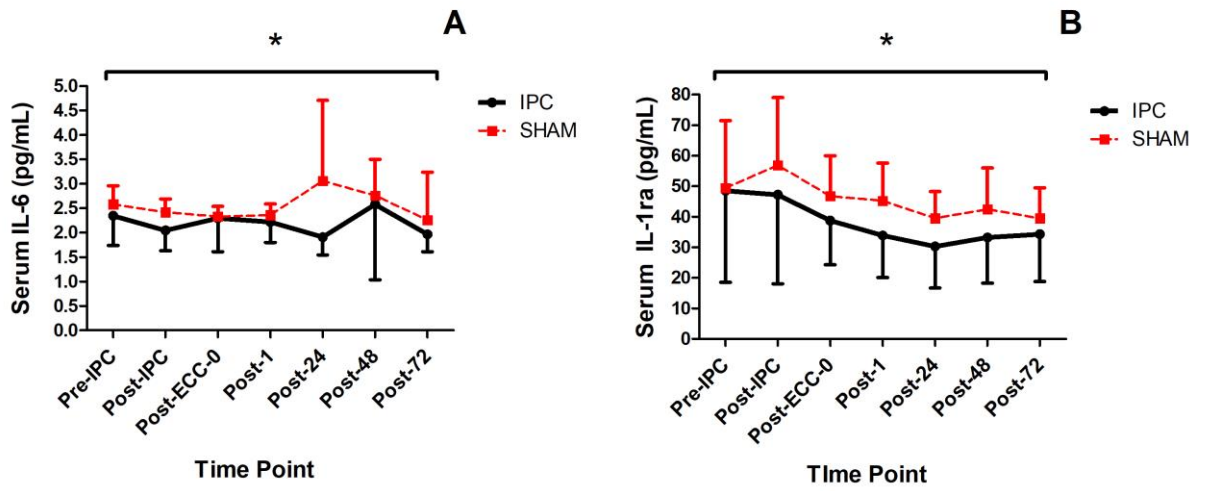


Figure 5. 4. Extracellular serum IL-6 (A) and IL-1ra (B) cytokine response (pg/mL) at individual time points in IPC and SHAM conditions. \* represents significantly lower serum cytokine levels between conditions.

### 5.3.6 Heat Shock Proteins (ng/mL)

There was a condition x time interaction in HSP-27 and HSP-32 ( $P<0.02$ , respectively). IPC resulted in greater serum levels of HSP-27 [1.01 (0.55, 1.47) ng/mL;  $P<0.005$ ] with significantly greater serum HSP-27 [3.01 (1.96, 4.06) ng/mL;  $P<0.005$ ] levels at post-IPC compared to SHAM (Figure 5.5). HSP-32 was also greater following IPC *versus* SHAM [0.13 (0.03, 0.23) ng/mL; main effect of condition:  $P=0.01$ ] with significant increases in HSP-32 observed Post-ECC-0 [0.32 (0.05, 0.57) ng/mL;  $P=0.02$ ], post-1 [0.38 (0.1, 0.63) ng/mL;  $P=0.005$ ] and at post-24 [0.37 (0.1, 0.63) ng/mL;  $P=0.007$ ] time points (figure 5.5). A main effect of condition was observed for HSP-72 ( $P=0.001$ ), with lower serum HSP-72 observed in IPC *versus* SHAM ( $P<0.005$ ). Reductions in HSP-72 were observed at pre-IPC in IPC *versus* SHAM (-0.44 (-0.81, -0.07;  $P=0.02$ ; figure 5.5) but HSP-72 was not different between



conditions at the post-IPC time point. Following eccentric exercise, HSP-72 was similar between conditions. Beyond this time-point, the gradual reduction in HSP-72 in IPC resulted in significantly lower values *versus* SHAM at post-48 and post-72 time points ( $P=0.007$  &  $P=0.004$ , respectively). There was a main effect of time for HSP-27, HSP-72 and HSP-32 ( $P<0.003$ , respectively). No condition x time interaction effect was observed for HSP-72 ( $P>0.05$ ).

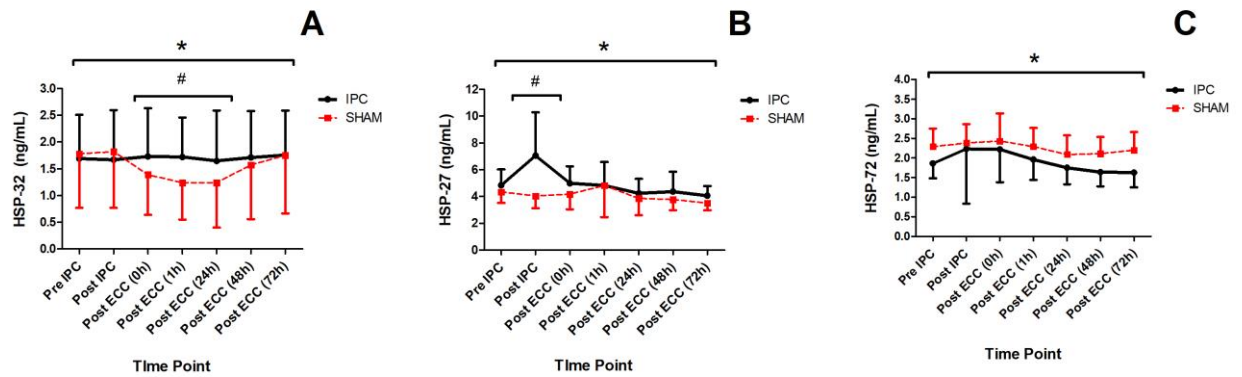


Figure 5. 5. Extracellular serum HSP-32 (ng/mL) (A), HSP-27 (ng/mL) (B) and HSP-72 (ng/mL) (C) responses at individual time points in IPC and SHAM conditions. \* represents statistically significant main effect of condition. # represents a condition x time interaction effect of IPC *versus* SHAM.

## 5.4 Discussion

The aim of this study was to investigate whether IPC could enhance muscle torque production (i.e. a measure of muscle function) following EIMD in humans and whether IPC mediated its effect on EIMD via a reduction in systemic cytokine and HSP stress responses. The following findings were evident. First, fixed angle voluntary MVC was enhanced at pre-ECC following IPC and this effect was maintained throughout the EIMD (0-72 hours) window *versus* SHAM. Second, performance of the slower ( $60 \text{ degrees} \cdot \text{sec}^{-1}$ ) dynamic contraction task was enhanced at pre-ECC following IPC compared to SHAM, an effect that was also present up to 72h post

EIMD. These effects were not present during the faster (120 deg.sec<sup>-1</sup>) dynamic contraction task. Third, reductions in extracellular cytokine responses to EIMD were observed, alongside alterations in HSP responses after IPC, when compared to SHAM. Taken together, these data provide preliminary evidence that IPC enhances torque production following EIMD, and this increase in skeletal muscle function, coincides with changes in both cytokine and HSP responses.

#### **5.4.1 Muscle Function Responses**

The current study, to the author's knowledge is the first study in humans to show that IPC enhances muscle torque production during maximal fixed angle and slower (60 degrees.sec<sup>-1</sup>) velocity dynamic contractions, before and after exposure to EIMD, when compared to a SHAM condition. It has been shown previously, using an animal model, that IPC can have a positive impact on skeletal muscle function, assessed by a 6-second maximal electrical stimulation task, in response to damage caused by 60 min of limb ischaemia (Phillips *et al.*, 1997) and these finding broadly support this data. The capacity for IPC to enhance muscle torque production prior to eccentric exercise in the current study also corroborate previous findings demonstrating IPC improved 5-repetition maximal isokinetic dynamometry when compared to SHAM in non-damaged muscle (Paradis-Deschênes *et al.*, 2016). Presently, explanation as to why IPC did not have a notable impact on the faster dynamic task (120 deg.sec<sup>-1</sup>) is unclear. The speed of movement during isokinetic tasks may induce differential functional responses on the muscle. Slower dynamic movement velocities (i.e. 60 deg.sec<sup>-1</sup>) are more appropriate to elicit peak torque (Baltzopoulos and Brodie, 1989), and may further prevent limb blood flow (Paradis-Deschênes *et al.* 2016), and increase intramuscular pressures (Saltin *et al.*, 1998) compared to faster velocity exercise tasks. Regardless, current findings suggest IPC has an overall beneficial response on muscle torque production pre- and post-EIMD in humans.

IPC can induce tissue protection during specific timing windows (Hausenloy and Yellon, 2010; Pang *et al.*, 1995). The timing of IPC administration may influence EIMD responses. IPC has been shown to induce immediate protection against ischaemic insult in skeletal muscle (Pang *et al.*, 1995), whereby 3x10 minute ischaemia/reperfusion cycles resulted in preferential maintenance of muscle metabolites following 4 hours of ischaemic insult in latissimus-dorsi muscle. This response was maintained following 1.5 hours of reperfusion (Pang *et al.*, 1995). Within the current study, all muscle function tests on the first experimental visit (day 1) were performed within this immediate (< 4 hour) protective window. Further tissue-protection can also be evident during a secondary protective timing window termed the second window of protection (SWOP) (Hausenloy and Yellon, 2010). Muscle function tests at 24-, 48- and 72-hours post eccentric exercise in the current study, coincided with the second window of protection (SWOP), and again, greater torque values were observed in this window.

#### **5.4.2 Extracellular Cytokine Responses**

Current study findings show reductions in both IL-6 and IL-1ra immediately after IPC (pre-ECC), which remained lower compared to SHAM after the exercise bout, possibly implying a blunted pro-inflammatory activity after eccentric exercise. IL-6 is thought to be an important mediator in the repair process following injury through its ability to inhibit expression of other pro-inflammatory cytokines such as IL-1 and TNF-alpha (TNF- $\alpha$ ) (Chamberlain *et al.*, 2013). IL-6 may exert greater influence on anti-inflammatory cytokines following EIMD, compared with IL-1ra (Peake *et al.*, 2005), however, IL-6 can promote expression of IL-1ra, a receptor agonist that directly antagonizes pro-inflammatory cytokine responses via binding to IL-1 receptors (Chamberlain *et al.*, 2013). The extracellular reduction in both IL-1ra and IL-6 markers after IPC *versus* SHAM suggests a lower pro-inflammatory response to eccentric exercise when preceded by IPC.

### 5.4.3 HSP responses

Induction of HSP-27 is likely a contributing factor in cellular protection afforded prior to prolonged ischemic insult in the myocardium (Das *et al.*, 1993), whilst translocation of small HSP's (HSP-25 & HSP-27) is specific to damaging (eccentric) contractions in animals (Koh and Escobedo, 2004). The results of the current study imply an enhanced HSP-27 response directly after IPC (pre-ECC); a finding consistent with previous work demonstrating enhanced HSP-27 mRNA after IPC in myocardium (Das *et al.*, 1993) and extracellular increases in HSP-27 release following global ischaemia (Jin *et al.*, 2014). Whether greater extracellular HSP-27 after IPC afforded greater tissue-protection against eccentric exercise in the current study is unknown. Regardless, this finding may represent one of many humoral responses that can potentially afford tissue protection against cellular damage.

HSP-32 is often expressed in conditions of oxidative challenge. This HSP is also described as Heme-oxygenase-1 (HO-1) and has been described as a distal mediator of delayed preconditioning in cardiomyocytes (Hausenloy and Yellon, 2010) whereby increased levels of HO-1 are observed during the SWOP after IPC (Jancso *et al.*, 2007). The current data show EIMD induced a reduction in HSP-32 in SHAM, but not in IPC, as IPC appeared to maintain HSP-32 levels. These findings may indicate that IPC provided greater anti-oxidant reserve status following EIMD *versus* SHAM (Stagos *et al.*, 2015), however anti-oxidant reserve status was not directly measured. Future work may help to elicit more detailed information surrounding this postulated mechanism.

In contrast to HSP-32, reductions in specific HSPs is purportedly due to a lower pro-oxidant state (Fehrenbach *et al.*, 2000) and in contrast to its intracellular molecular chaperone role, extracellular HSP-72 acts as a potent cytokine, stimulating pro-inflammatory mediators after incurring stressful stimuli (Asea *et al.*, 2000). The overall main effect of lower extracellular

HSP-72 in IPC *versus* SHAM conditions may have been partly influenced from lower pre-IPC (baseline) HSP-72 in the IPC condition. Nevertheless, HSP-72 immediately increased following after application of IPC, to match SHAM levels. Gradually, HSP-72 reduced following EIMD in the IPC condition and significant differences were observed at post-48 and post-72-time points. In the current study, the extracellular elevations in HSP-27 and HSP-32, but not HSP-72 after EIMD, may suggest IPC blunts the systemic stress response to a bout of eccentric exercise.

#### **5.4.4 Limitations**

All cytokine and HSP analyses were performed on extracellular serum concentrations. The roles of extracellular vs intracellular stress responses vary greatly, so the current data should be interpreted with caution. Another limitation is that cytokines or HSP responses are affected by the magnitude of oxidative stress incurred following EIMD, a variable known to vary between individuals (Stagos *et al.*, 2015). However, the crossover design of this study, in combination with the 9-week washout, controlled for inter-individual variation in cytokine and HSP responses. Therefore, it is likely that this does not impact the main study findings. To date, there is no consensus on the wash out period needed to abolish the repeated bout effect. The repeated bout effect is characterized by a blunted damage response to eccentric exercise when a previous eccentric exercise bout has been performed. There are suggestions that the repeated bout effect can persist up to 6-months after EIMD (Nosaka *et al.*, 1991). Importantly, the order of testing blocks (IPC/SHAM) was randomized. Previous data shows serum stress biomarker and perceived muscle soreness responses to EIMD are suppressed at 3- and 6-weeks post-exercise (Byrnes *et al.*, 1985; Nosaka *et al.*, 1991), therefore a 9-week washout period was employed in the current study. Previous work has demonstrated significant muscle damage responses after the exact protocol used in the current study (Owens *et al.*, 2015b) and, the reduction in both DOMS ratings and muscle torque were similar to values reported elsewhere (Stagos *et al.*,

2015). Based on the above, it is likely that sufficient muscle damage was induced prior to measurement of both muscle function and extracellular stress responses.

#### **5.4.5 Conclusion**

The findings of the current study suggest that IPC enhances torque production in non-damaged muscle *versus* SHAM. EIMD resulted in similar decrements in muscle torque in both IPC and SHAM conditions, however, IPC maintained greater torque production capability throughout the (<72 hr) EIMD window alongside favourable changes in cytokine and HSP responses. These findings may suggest IPC can elicit favorable damage responses in recreationally trained humans that experience EIMD.

## Chapter 6

A comparison of ischaemic preconditioning and muscle heat maintenance as exercise priming strategies

## 6.1 Introduction

Priming/preconditioning methods, administered prior to performance, aiming to augment or potentiate high-intensity exercise performance are now common practice in elite sport (Beaven *et al.*, 2018). Most athletes will aim to sufficiently warm-up before competition, but certain priming strategies may magnify the benefits of warm-ups, including ischaemic preconditioning (IPC) (Patterson *et al.*, 2015), and muscle and/or core temperature manipulation (Beaven *et al.*, 2018; Kilduff *et al.*, 2013b).

Ischaemic preconditioning, administered at an appropriate time prior to exercise performance has previously been shown to enhance both aerobic (Bailey *et al.*, 2012; Crisafulli *et al.*, 2011; Kido *et al.*, 2015) and anaerobic (Cruz *et al.*, 2016; Paradis-Deschênes *et al.*, 2016; Patterson *et al.*, 2015) exercise performance. The proposed mechanisms contributing to performance enhancements include enhanced  $\dot{V}O_2$  kinetics (Kido *et al.*, 2015), greater muscle oxygenation and perfusion (Paradis-Deschênes *et al.*, 2016) and enhanced neuromuscular responses to exercise (Cruz *et al.*, 2015). Therefore, IPC could be an effective strategy to improve exercise performance that is dependent on both anaerobic and aerobic capacity, such as repeated sprint ability.

Performance improvements have also been observed when muscle temperature is elevated prior to exercise performance. Specifically, sprint-based exercise can benefit from higher muscle temperature at the beginning of exercise (Beaven *et al.*, 2018; Faulkner *et al.*, 2013a; Kilduff *et al.*, 2013b). Increased muscle temperature is thought to improve performance via a range of mechanisms, such as, enhanced muscular function at the onset of maximal exercise (Ferguson *et al.*, 2002) and improved muscle contraction velocity capacity and rate of force development (Racinais and Oksa, 2010; Ranatunga, 1982).

Taken together, both IPC and local muscle heating strategies can have a significant impact on sprint-type exercise, but the mechanisms mediating these performance improvements are likely



different. IPC, unlike localised muscle heating has also shown benefit to aerobic-type performance in humans (Salvador *et al.*, 2016). Therefore, combining the priming/preconditioning strategies may be additive to RSA performance, especially as RSA tasks are highly taxing of both aerobic and anaerobic energy systems (Girard *et al.*, 2011). The primary aim of this study was to examine whether IPC, passive local muscle maintenance, or a combination of both strategies during warm-up improves repeated sprint ability (RSA) cycling performance. The secondary aim of this study was to relate any changes in performance (power output) with muscle oxygenation and muscle activation responses in trained cyclists.

## 6.2 Methods

### 6.2.1 Location of testing and Ethical Approval

All experimental protocols and data collection procedures were undertaken in the research testing laboratory of the Athlete Health and Performance laboratory, situated inside Aspetar Sports Medicine Hospital, Doha. All studies included in this thesis were presented to and approved by both the local chief medical officer scientific committee and the local ethics committee (Anti-Doping Laboratory Qatar; ADLQ). Ethical approval number for this study was F2017000217.

### 6.2.2 Inclusion/exclusion criteria

Physical Activity Readiness Questionnaires (PAR-Q) were administered to ensure no participant had no contraindication that would prevent participation. Participants were included in the study if they were a competitive cyclist that had sprint training experience and had a  $\dot{V}O_{2\max}$  above  $50\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$ . Participants were excluded if they answered “yes” to any PAR-Q question, or if they had recent history of an injury that lead to a detraining period of more than

1-week. Exercise was prohibited 48 hours prior to testing, whilst caffeine and alcohol were restricted at least 6 and 24 hours prior to laboratory testing, respectively.

### **6.2.3 Participants**

Eleven trained males (mean $\pm$ SD: age, 33.7 $\pm$ 4.8 years; body mass, 81.1 $\pm$ 10 kg; height 181 $\pm$ 7 cm;  $\dot{V}O_{2\max}$ , 57.4 $\pm$ 5.0 ml $\cdot$ kg $\cdot$ min $^{-1}$ ) undertaking regular high intensity interval cycling training (1-2 sessions per week) were recruited. Mean training experience was 9 $\pm$ 8 years and mean weekly cycling training volume was 7 $\pm$ 3 hours.

### **6.2.4 Research Design**

The study compared four preconditioning strategies, as displayed in figure 6.1. Prior to commencement of four Repeated Sprint Ability (RSA) cycling tests. A practical pre-competition model was employed that included 4 different preconditioning strategies; (1) IPC and thermoneutral (T-N) lower body insulation (IPC), (2) IPC and passive muscle heating of the lower limbs (IPC+HEAT), (3) SHAM and T-N lower body insulation (SHAM) and (4) SHAM and passive muscle heating of the lower limbs (HEAT). The four preconditioning strategies were administered in a randomized counterbalanced order.

### **6.2.5 Experimental Protocol**

Prior to experimental visits, all participants completed familiarisation sessions and a maximal graded cycling test to establish  $\dot{V}O_{2\max}$  and watt max ( $W_{\max}$ ). During the experimental visits, participants entered the laboratory and firstly performed either ischaemic preconditioning (IPC) or a control (SHAM). Immediately following IPC or SHAM, in thermoneutral (T-N) conditions, insulation (elasticated tubigrips) was administered on the lower limb musculature. In heated (heated) conditions, 16 x heated pads (secured with elasticated tubigrips) were placed on the lower limb musculature and participants rested for 20-minutes. A 19-minute standardised cycling warm-up was then performed whilst wearing the insulation on the lower limbs. The

warm-up protocol aimed to elevate muscle temperature to above 39 degrees Celsius (°C) in both the T-N and heated conditions (see figure 6.2). After the cycling warm up participants rested for a further 12 minutes with the lower limb insulation still attached (either T-N or heated). During this period electromyography (EMG) electrodes (x3) and near-infrared spectroscopy (x1) probes were placed on the left and right leg, respectively. At minute 14 post-warm up, participants remounted the cycle ergometer and began pedaling at approximately 75 watts (W), aiming to reach a cadence of 105 revolutions per minute (RPM) within 1-minute. At minute 15 post-warm up, a 10x6-second repeated sprint task commenced. All participants were instructed to elicit peak power for the entire duration of each 6-second effort. Sprints were repeated 10 times interspersed with 24-seconds of recovery (9 seconds stationary recovery; 15 seconds spinning at 75 W). The primary performance outcomes were peak and average power output. Sprint-by-sprint muscular activation and muscle oxygenation properties were secondary measures.

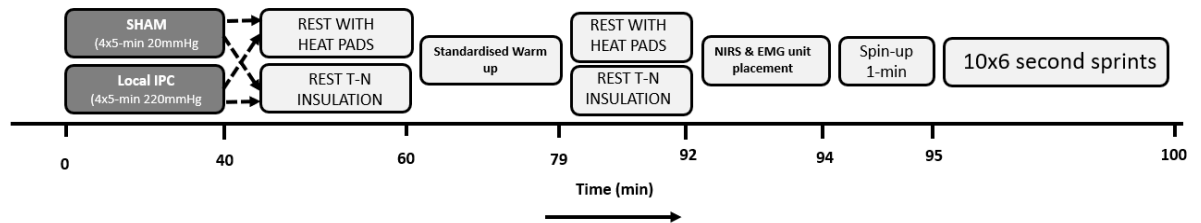


Figure 6. 1. Schematic of experimental protocol detailing the order of events on each laboratory visit

## 6.2.6 Preliminary assessments

### 6.2.6.1 Assessment of maximal oxygen uptake ( $\dot{V}O_{2max}$ )

At least 7 days prior to the first familiarisation session, participants performed a continuous incremental step test on a cycle ergometer (SRM, Julich, Germany) to determine maximal oxygen uptake ( $\dot{V}O_{2max}$ ). The protocol consisted of 3-minute stages and commenced at a power output of 95 W. An increase in 35 W per step occurred until volitional exhaustion was attained. Throughout the incremental test, breath-by-breath expired gases were monitored for oxygen consumption, ventilation and respiratory exchange ratio (Oxycon Pro™, Carefusion, Germany) and the highest 30-second average, determined from 3 consecutive 10-second bins was taken to determine  $\dot{V}O_{2max}$ . Heart rate was also monitored continuously (Polar HI, Kempele, Finland). Maximal power output ( $W_{max}$ ) was calculated from the last completed workload, plus the fraction of time spent in the final non-completed stage multiplied by the work rate increment (Jeukendrup *et al.*, 1996).

#### 6.2.6.2 Familiarisation.

At least 2 familiarisation trials of 10x6-second all-out sprints, separated by 24 seconds of recovery, were undertaken prior to experimental visits. This determined the natural variation in participant performance. Further familiarisation sessions were implemented if necessary. Data from familiarisation testing revealed coefficient of variations of  $2.5\pm 1.7\%$  &  $3.1\pm 1.5\%$  for mean and peak session power over the 10x6-second repeated sprints, respectively.

### **6.2.7 Experimental Visits**

#### 6.2.7.1 IPC protocols.

For both the IPC and SHAM preconditioning strategies, a 13.5 cm wide cuffs were placed bilaterally on the most proximal portions of the upper thighs. Participants lay in a supine position and cuff inflation pressure was standardised at 220 mmHg for the IPC condition and 20 mmHg in the SHAM condition, respectively. Occlusion was attained with use of an automatic rapid cuff inflator (Hokanson, Washington, USA). Occlusion occurred for a duration of 5-minutes, interspersed with 5-minutes of complete deflation (0 mmHg). This process was repeated 4 times in total.

#### 6.2.7.2 Skin Temperature.

Immediately following IPC or SHAM, a skin temperature sensor/data logger (iButton<sup>TM</sup>, Maxim Integrated Products, Sunnyvale, CA USA) was activated and time synchronized prior to being placed on the upper right thigh. Skin temperature was continuously recorded throughout the duration of the experimental visits. Skin temperature data was downloaded at a sampling rate of 60 (1 sample per minute) and exported to CSV file. Data was analysed at seven specific time points (1: Start warm up, 2: Warm up 5-min, 3: Warm up 10-min, 4: Warm up 15-min, 5: Finish warm up (Warm up 19-min), 6: Start Sprint & 7: Finish Sprint).

### 6.2.7.3 Lower Limb Insulation.

After placement of the skin temperature button, lower limb insulation was placed on the lower limbs. In heated (HEAT and IPC+HEAT) conditions, sixteen (8 pads per limb) air activated adhesive muscle heat pads (THE HEAT COMPANY GmbH, Altenmarkt, Austria), reaching approx. 43°C in temperature were placed on the lower (VM, VL, RF, Gastroc, Soleus) limbs and secured with elasticated tubigrips (12cm Tubular support bandage, Bastos Viegas, s.a. Guilhufe, Penafiel-Portugal). For T-N (IPC and SHAM) conditions, no muscle heat pads were used, instead, elasticated tubigrips were placed directly on the skin.

### 6.2.7.4 Cycling Warm-Up

The warm-up was conducted on a cycle ergometer (SRM, Julich, Germany) lasting 19 minutes, 10 seconds in total. The  $W_{\max}$  value attained from the incremental test was used to prescribe 3 submaximal warm up stage intensities. These stages consisted of 5-minutes at 40, 50 & 60%  $W_{\max}$  respectively. At minute 15 of the warm up, participants increased cadence to 105 RPM, and at 15:30, began a progressive sprint warm up task consisting of 2 x 5 second efforts @ 50% peak power, 2 x 5 second efforts @ 75% peak power & 2 x 5 second efforts @ 100% peak power. The 50%, 75% and 100% 5-second efforts were separated by 10, 30 and 60 seconds of rest, respectively. Heart rate was monitored throughout the warm up (Polar H1, Kemple, Finland).

#### *Validation of Muscle Temperature Maintenance:*

To ensure the cycling warm up elicited a sufficient increase in muscle temperature in T-N and heated conditions, intramuscular muscle temperature was recorded via indwelling muscle temperature probes in two participants (Figure 6.2) prior to commencement of the study. One leg was heated with heat pads, while the other leg replicated T-N conditions. Muscle temperature was recorded during a simulated warm-up exercise bout and then monitored during the 15-

minute recovery period. On a separate day to validating muscle temperature, skin temperature was also monitored. After 30-minutes of heat pad application on a single leg, skin temperature was continuously monitored for 5.5 hours on both heated, and non-heated legs. Mean skin temperature value for the heated leg was  $41.2 \pm 0.8$  °C versus  $32.7 \pm 1$  °C for the non-heated leg.

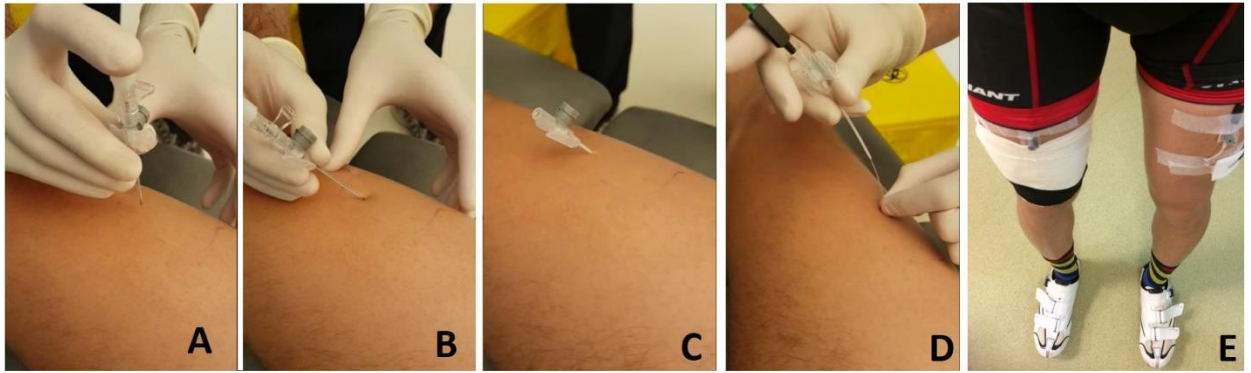


Figure 6. 2. Muscle temperature assessments used to validate the warm-up protocol prior to experimental testing. (A) Located at 2/3 of Vastus lateralis from anterior superior iliac spine to lateral patella (B) the canula was aligned with the fiber pennation angle (C) and inserted prior to (D) inserting a muscle temperature probe at a depth of 2.5cm. Finally (E) after this procedure was repeated on both legs, participants mounted the bike with one heated quadriceps (2 x muscle heat pads) and one T-N (non-heated) leg and began the warm up. Muscle temperature of each leg was recorded at set time points and is detailed below.

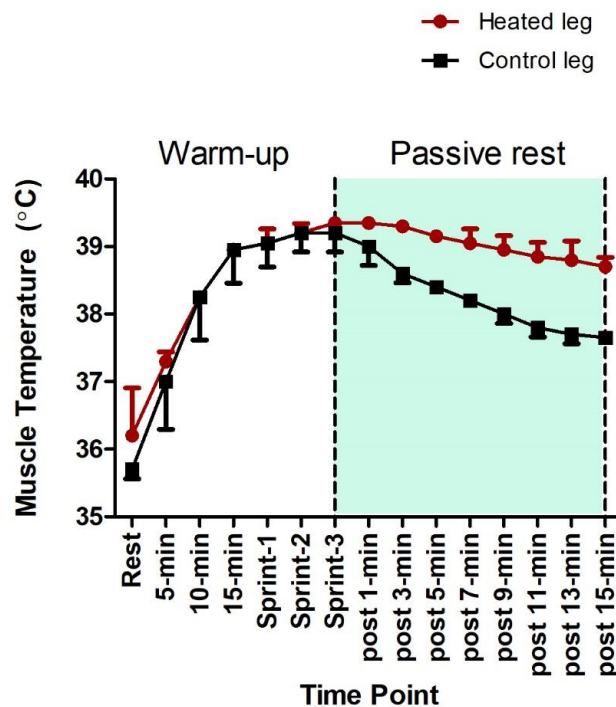


Figure 6. 3. Muscle temperature (depth at 2cm) data collected to prior to experimental trials, to validate the muscle heat maintenance protocol during and after the cycling warm-up. Data were collected throughout warm-up and 1-15-minutes post warm up. Muscle temperature at the end of the warm-up equaled  $39.4 \pm 0.1$  °C in heated leg and  $39.2 \pm 0.3$  °C in control leg (N=2).

#### 6.2.7.5 Muscle Heat Maintenance

As soon as the warm up was completed, participants dismounted the bike and returned to a supine resting position for a total of 14-minutes, with either the application of muscle heat pads, or T-N insulation.

#### 6.2.7.6 Near Infrared Spectroscopy

Changes in muscle oxygenation during the RSA test was determined using a wireless, continuous-wave near infrared spectroscopy (NIRS) system (Portamon; Artinis Medical Systems, BV, The Netherlands). The Portamon simultaneously uses the modified Beer-Lambert and spatially-resolved spectroscopy methods to determine changes in oxygenated haemoglobin ( $\Delta O_2Hb$ ) and de-oxygenated haemoglobin ( $\Delta HHb$ ), expressed in micromolar units ( $\mu M$ ). Due to spectral similarities, the contribution of myoglobin to the NIRS signal cannot be differentiated but is believed to be minimal (Ferrari *et al.*, 2004; Mancini *et al.*, 1994). This system also provides a measure of  $O_2Hb$  saturation indicated by the tissue oxygenation index [TOI (%)], which reflects the dynamic balance between  $O_2$  demand and supply within the muscle microcirculation (McCully and Hamaoka, 2000). The Portamon unit consisted of three emitter diodes positioned 30, 35, and 40 mm from the detector, and emitted infrared light at wavelengths of 760 and 850 nm. All analyses were undertaken on data gathered from the 35 mm emitter-detector distance, corresponding to a NIRS signal penetration depth of approximately 17.5 mm (Ferrari *et al.*, 2011; McCully and Hamaoka, 2000). The Portamon unit was secured on the right limb vastus lateralis (VL) muscle using black adhesive tape (Kinesio Co., Ltd, Tokyo) reinforced with elasticated Tubigrip to prevent movement and signal contamination from



external light sources. The probe site was standardised for accurate re-positioning; at 2/3 of the distance from the anterior superior iliac spine (ASIS) to the lateral patella. The area of investigation was lightly shaved with a safety razor (Double Edge shaving Comb razor, National Medical Products Co. Ltd. KSA) and cleaned with a disposable sterile isopropyl alcohol swab (China Meheco Co, Ltd) prior to probe placement. All NIRS data were collected at 10 Hz using a dedicated software (Oxysoft, Artinis Medical Systems) and down sampled to 1 Hz for further analyses. Finally, a differential pathlength factor of 4 was implemented to account for scattering of light signal. A description of the analysis procedure is provided in figure 6.4.

#### 6.2.7.7 NIRS Data Analysis

Typical changes in TOI during repeated sprint cycling are presented in Figure 6.7 of results section. A rapid decrease in TOI (i.e., muscle de-oxygenation) was observed following the onset of sprinting. During rest intervals, the recovery in TOI was preceded by a time delay (TD), which was the corresponding time (s) between the end of the sprint and the lowest 1-s TOI value before sustained re-oxygenation was observed (refer to shaded region in Figure 6.4). Muscle deoxygenation amplitude ( $Deoxy_{AMP}$ ) was determined by the change in TOI during each sprint (i.e.,  $TOI_{max} - TOI_{min}$ ), where  $TOI_{max}$  and  $TOI_{min}$  refer to the maximum and minimum TOI values at the onset, and at the end of each sprint, respectively (refer to figure 6.4). Similarly, re-oxygenation amplitude ( $Reoxy_{AMP}$ ) was determined by the change in TOI during recovery ( $recTOI_{max} - recTOI_{min}$ ), where  $recTOI_{min}$  and  $recTOI_{max}$  refer to the lowest and highest TOI values within the 10-s recovery period following the sprint. Muscle de-oxygenation and re-oxygenation rates (following TD) were determined via simple regression as previously described (Ihsan *et al.*, 2013):  $TOI = a \times t + b$ , where  $a$  is the slope ( $\% \cdot s^{-1}$ ),  $t$  is the time (s) and  $b$  is the y-intercept (%). The slope ( $a$ ) of the linear model fit was retained as the index of muscle de-oxygenation ( $Deoxy_{slope}$ ) or re-oxygenation ( $Reoxy_{slope}$ ) rates (figure 6.4). Linear modelling and analysis using minimum and maximum values demonstrates the best reliability for muscle

de-oxygenation and re-oxygenation rates (Ihsan *et al.*, 2013), with previously reported coefficients of variation for  $Deoxy_{SLOPE}$ ,  $Reoxy_{SLOPE}$  and TD of 7.2%, 21.4% and 8.1%, respectively (Ihsan *et al.*, 2013).

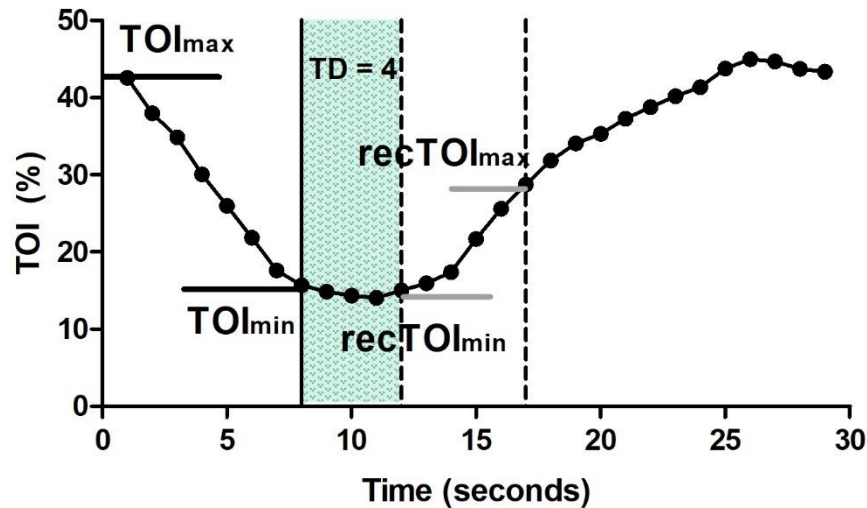


Figure 6. 4. Example of a single 6-second sprint, followed by a 24-second recovery. Assessment of NIRS data during sprints were obtained from the first 7 seconds of effort, accounting for any additional pedal strokes. Deoxygenation amplitude ( $Deoxy_{AMP}$ ) and slope ( $Deoxy_{slope}$ ) were calculated from the maximal ( $TOI_{max}$ ) and minimal ( $TOI_{min}$ ) values (black horizontal lines). Time Delay (TD) was defined as the time (in seconds) from post-sprint to the beginning of muscle reoxygenation. Recovery amplitude ( $Reoxy_{AMP}$ ) and rate ( $Reoxy_{slope}$ ) were calculated from maximal ( $recTOI_{max}$ ) and minimal ( $recTOI_{min}$ ) values (grey horizontal lines).

#### 6.2.7.8 Electromyography.

Skin was prepared during the rest interval after warm-up cessation (see “Near Infrared Spectroscopy” methods section) and site positioning was standardised for each of the three muscles of interest (Rectus Femoris: 50% from ASIS to patella border; Vastus Lateralis: 2/3 distance from ASIS to lateral patella; Biceps Femoris: 50% from ischial tuberosity to lateral patella). Electromyography was used to record and analyse myoelectric signals during sprint activity, whilst one electrode was placed on the cycle ergometer crank and used as an accelerometer. Electrodes were secured using black adhesive tape (Kinesio Co., Ltd, Tokyo)

and were reinforced with elasticated Tubigrip on the upper limb to prevent unit movement and signal contamination. All data were recorded at 2000 Hz using a Delsys Trigno Wireless System (Boston, MA), rectified and low-pass filtered with a Butterworth third-order low-pass zero-lag filter and a cut-off frequency of 20 Hz. To assess relative muscle activation across the different sprints, the EMG signal was normalized to its respective maximal contraction value obtained during the 10 sprints for each participant and condition and the respective peak amplitude calculated. To account for slight variation in placement between experimental visits and signal variance, a root mean squared (RMS) value, which reflects the mean power of the EMG signal, recommended for smoothing EMG data, was calculated for each sprint effort to derive muscle activation. The maximum RMS value for each muscle group was classified as 100%, all other RMS values were normalized to this value (% of maximum RMS).

#### 6.2.7.9 Repeated Sprint Ability Test

During the repeated sprint protocol, the cycle ergometer (SRM, Julich, Germany) was set to isokinetic mode, whereby cadence was clamped at 105 RPM. Participants were instructed to build cadence to 105 RPM in the first minute, prior to commencing sprint 1. Participants completed 10 maximal sprint efforts in total, with each sprint lasting 6-seconds in duration with 24-seconds of passive recovery in between. They were instructed to produce ‘all out’ effort from time point 0 until the end of the sprint. Participants began building cadence 15 seconds prior to each sprint to ensure cadence was at 105 RPM for the start of each repetition.

#### 6.2.7.10 Power Output Calculations

Sprint power output was determined by analyzing SRM ergometer sprint files via custom training software (Golden Cheetah, version 3.3). Files were selected, and a custom interval search was conducted to find the 10 highest 6-second power output bouts for each test. Average and peak power output values were recorded for each sprint.

### 6.2.8 Statistical Analysis

Using a magnitude-based inference framework, the mean effect of each sprint comparison was presented for the primary outcomes, with uncertainty of the estimates presented as 90% confidence intervals (appropriate SI units for a given variable). The mean difference between each comparison were evaluated for their practical significance by pre-specifying the smallest worthwhile change (SWC) (Batterham and Hopkins, 2006). For session average power output, and session peak power output, the SWC was calculated using  $0.3 \times$  coefficient of variation from familiarisation data (Hopkins *et al.*, 2009), which equated to 6 and 9 watts, respectively. The noise to signal ratio was determined by calculating the typical error (TE; the SD of between-trial differences divided by  $\sqrt{2}$ ). The TE for peak and mean power output was 16 and 20 watts, respectively. Between each comparison, together with its uncertainty, the probability (percent chances) that the true population effect was beneficial ( $>SWC$ ), harmful ( $>SWC$  with opposite sign), or trivial (within  $\pm SWC$ ) was calculated (see *section 3.2.7* for details).

In combination with running magnitude-based inference statistics, the peak power and average (6-second) power output for each sprint, in addition to NIRS and EMG data for each sprint were analysed using a repeated measures 2-way general linear model (Condition [4 levels: IPC vs IPC + HEAT vs SHAM vs HEAT], Time [10 levels]: comparison of sprints 1-10) with 95% confidence intervals. The least significant (LSD) method was employed for pairwise comparisons (Perneger, 1998). All data were checked for normal distribution prior to statistical analysis, using the Shapiro-Wilk test.

## 6.3 Results

### 6.3.1 Average & Peak Power Output

IPC resulted in a 12 watts improvement (90% CI: -7, 31 watts) in average power output *versus* SHAM, which was greater than the SWC (6 watts) but lower than the calculated typical error (16 watts) and therefore was deemed an “unclear” change (Figure 6.5). HEAT *versus* SHAM [9 watts (90% CI: -9, 28)] and IPC *versus* IPC+HEAT [7 watts (90% CI: -11, 26)] also resulted in changes greater than the calculated SWC but again were lower than the calculated typical error so were reported as “unclear” (Figure 6.5). There was a significant decrease over time in both 6-second and peak power output ( $P < 0.005$ , respectively). The mean reduction in 6-second power output from sprint 1 to 8 was  $42 \pm 23$  watts and the mean reduction in peak power from sprint 1 to 7 was  $40 \pm 8$  watts ( $P < 0.006$ , respectively). Both 6-second power and peak power output plateaued at sprint number 9 and 8, respectively ( $P > 0.05$ , respectively). There was no effect of condition on either 6-second power ( $P = 0.64$ ) or peak power output ( $P = 0.98$ ) (Figure 6.6) across the 10 sprints and no condition x time interaction effect was observed for either peak ( $P = 0.67$ ) or 6-second ( $P = 0.3$ ) power output.

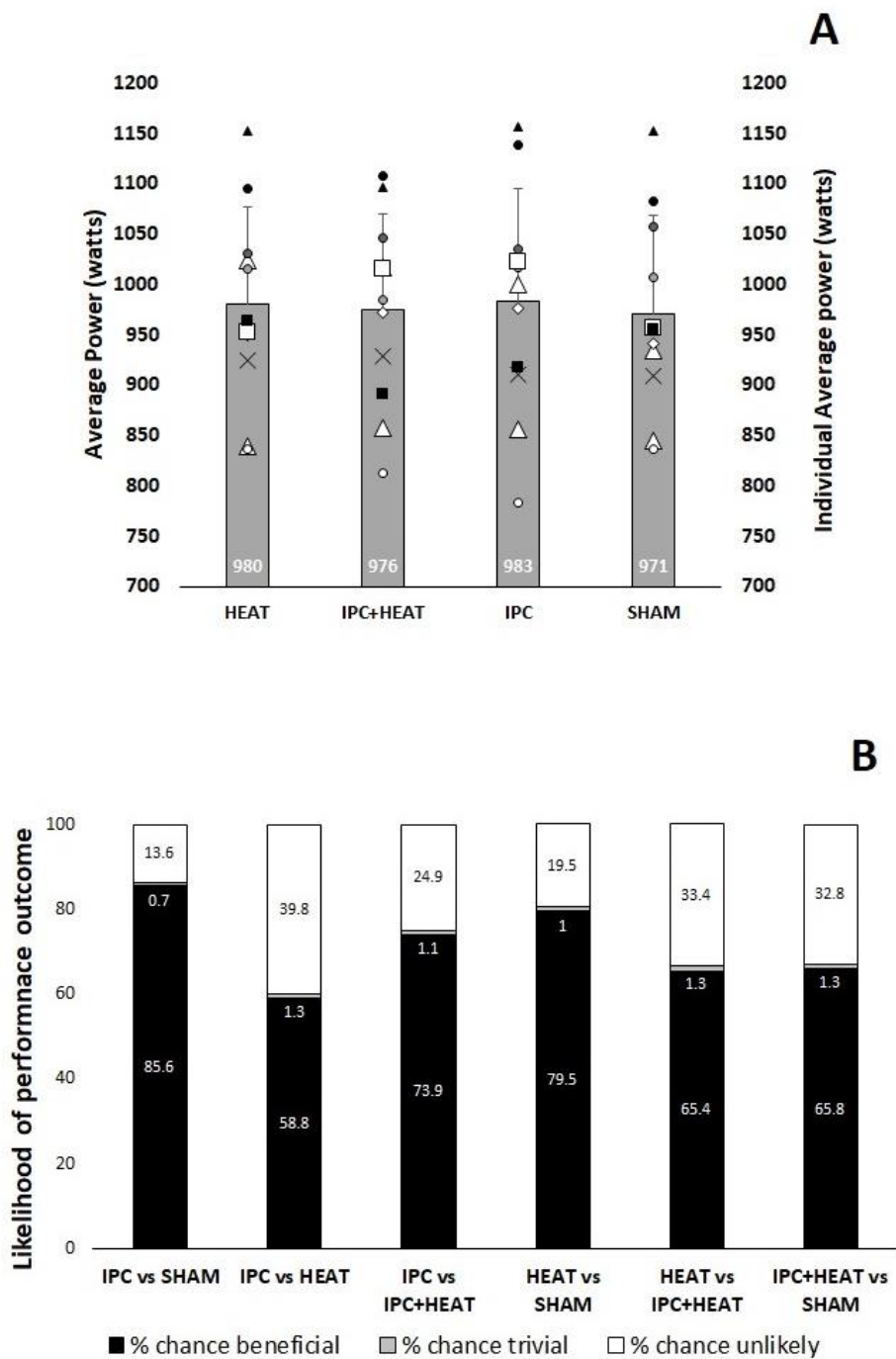


Figure 6. 5. Average power output during 10x6-second all out sprints between conditions (A), accompanied with likelihood of “beneficial”, “trivial” or “unlikely” performance outcome to 10x6-second average power output (B).

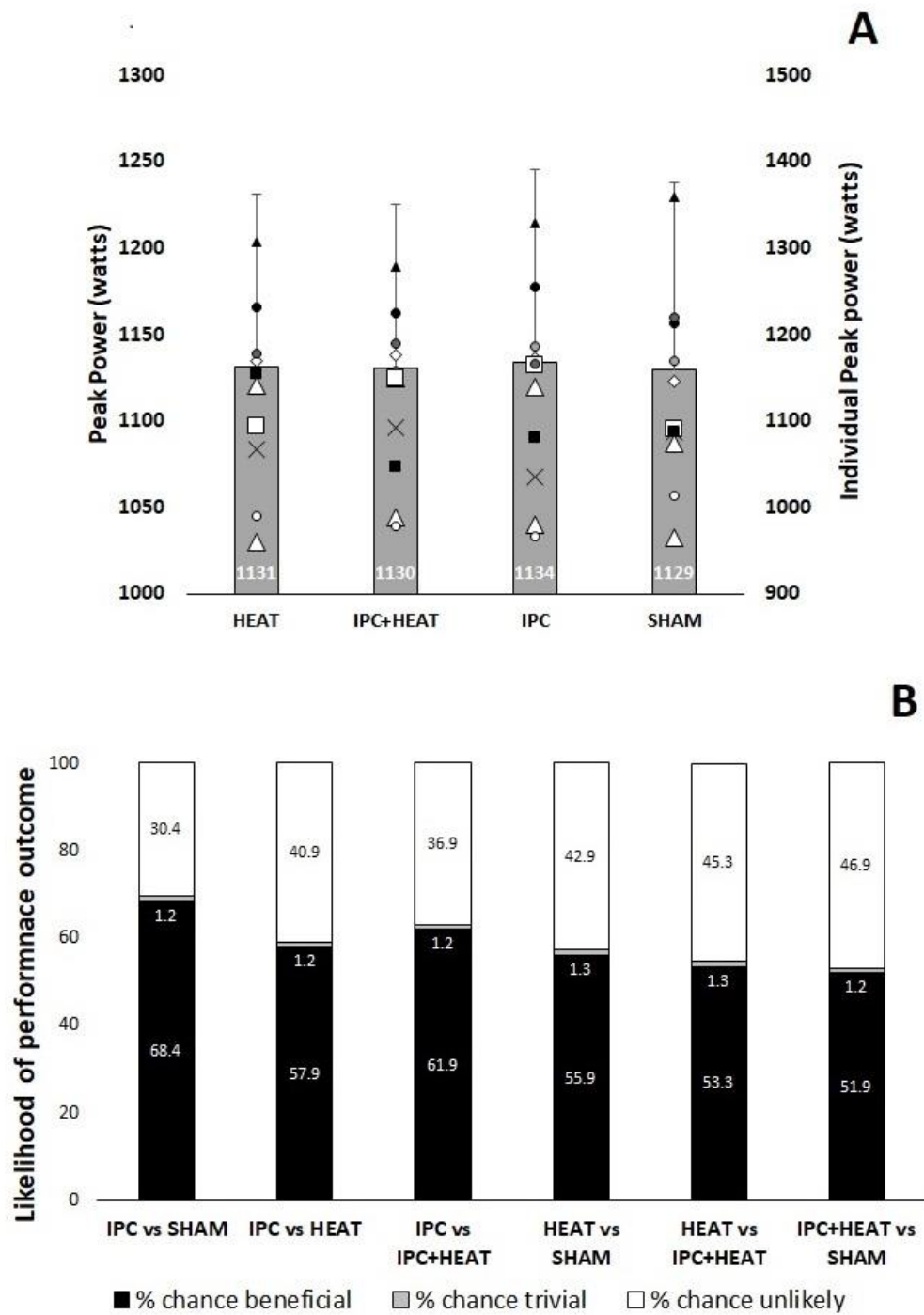


Figure 6. 6. Peak power output during 10x6-second all out sprints between conditions (A), accompanied with likelihood of “beneficial”, “trivial” or “unlikely” performance outcome to 10x6-second peak power output (B).

### 6.3.2 Muscle Deoxygenation

There was a significant reduction over time ( $P < 0.005$ , respectively) in  $\text{DeOXY}_{\text{AMP}}$  (%) and  $\text{DeOXY}_{\text{slope}}$  ( $\% \cdot \text{second}^{-1}$ ) with sprint 1 resulting in the largest  $\text{DeOXY}_{\text{AMP}}$  and  $\text{DeOXY}_{\text{slope}}$  when compared to all other sprints ( $P < 0.03$  &  $P < 0.006$ , respectively). There was no overall main effect of condition for either  $\text{DeOXY}_{\text{AMP}}$  ( $P = 0.11$ ) or  $\text{DeOXY}_{\text{slope}}$  ( $P = 0.08$ ). However, a trend for greater  $\text{DeOXY}_{\text{AMP}}$  after both HEAT and IPC+HEAT may have been present *versus* SHAM [4.5 (-0.9, 9.8) % & 4.8 (-0.7, 10.3) %;  $P = 0.1$  &  $P = 0.08$ , respectively]. SHAM resulted in a lower mean  $\text{DeOXY}_{\text{slope}}$  compared to both IPC+HEAT [0.77 (-0.001, 1.54);  $P = 0.05$ ] and HEAT [0.76 (-0.1, 1.63);  $P = 0.08$ ] conditions. No condition x time interaction effect was reported for either  $\text{DeOXY}_{\text{AMP}}$  or  $\text{DeOXY}_{\text{slope}}$  ( $P > 0.05$ , respectively).

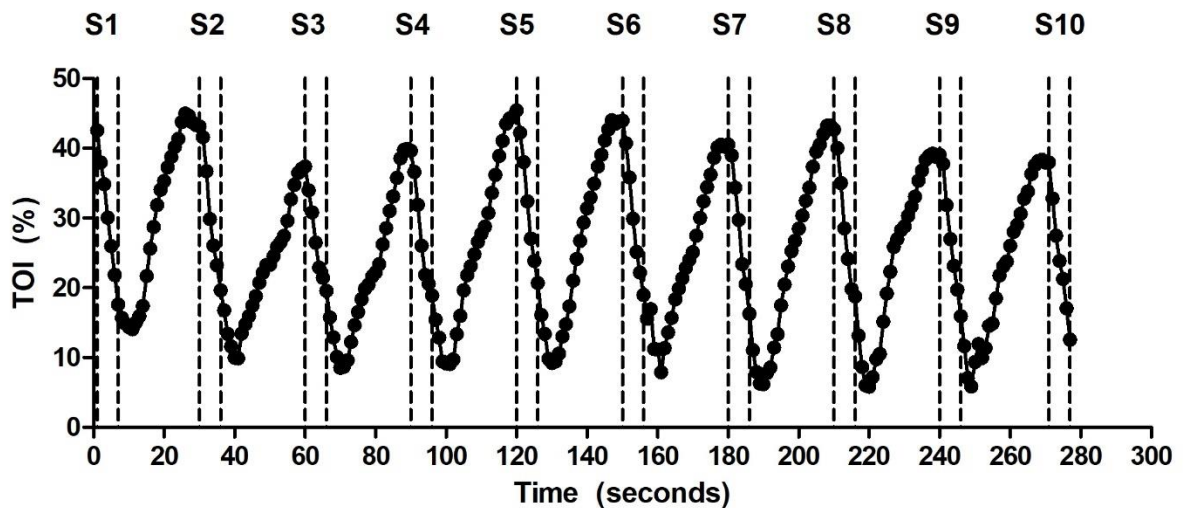


Figure 6. 7. Typical NIRS muscle oxygenation data during 10x6-second all out sprints. TOI; Tissue oxygenation index, S1-10; Sprint number.

### 6.3.3 Muscle Reoxygenation

There was no main effect observed for time for either  $\text{ReOXY}_{\text{AMP}}$  (%) ( $P = 0.08$ ) or  $\text{ReOXY}_{\text{slope}}$  ( $\% \cdot \text{second}^{-1}$ ) ( $P = 0.47$ ). No main effect for condition was observed for  $\text{ReOXY}_{\text{AMP}}$  ( $P = 0.18$  or



Reoxy<sub>slope</sub> ( $P=0.19$ ), respectively. IPC+HEAT showed a trend for greater (2.2 to 2.4 %) increases in Reoxy<sub>AMP</sub> compared to HEAT, IPC and SHAM preconditioning strategies ( $P=0.09$ ,  $P=0.19$  &  $P=0.07$ , respectively; Table 6.1), whilst IPC+HEAT also showed a statistical trend for greater Reoxy<sub>slope</sub> compared with SHAM ( $0.5\% \cdot \text{second}^{-1}$ ;  $P=0.05$ ). No condition x time interaction was observed for either Reoxy<sub>AMP</sub> or Reoxy<sub>slope</sub> ( $P>0.05$ , respectively).

Table 6. 1. Muscle deoxygenation [Deoxy<sub>AMP</sub> (%) & Deoxy<sub>slope</sub> ( $\% \cdot \text{second}^{-1}$ )] and reoxygenation [Reoxy<sub>AMP</sub> (%) and Reoxyslope ( $\% \cdot \text{second}^{-1}$ )] characteristics for 10 x 6-second repeated sprints

	<b>Deoxy<sub>AMP</sub></b>	<b>Deoxy<sub>slope</sub></b>	<b>Reoxy<sub>AMP</sub></b>	<b>Reoxy<sub>slope</sub></b>
<b>IPC</b>	15 ± 7	3 ± 1	8 ± 2	1.1 ± 1
<b>HEAT</b>	18 ± 8	3 ± 1	8 ± 3	1.4 ± 1
<b>IPC+HEAT</b>	19 ± 9	3 ± 1	11 ± 5	1.5 ± 1
<b>SHAM</b>	13 ± 4	2 ± 1	8 ± 3	1 ± 0

### 6.3.4 EMG RMS (%)

No time ( $P=0.17$ ), condition ( $P=0.55$ ), or condition x time interaction ( $P=0.57$ ) effect was observed for VL RMS. For both RF and BF, a significant reduction in RMS was observed over time ( $P=0.02$  &  $P<0.005$ , respectively). There was no main effect of condition for either RF ( $P=0.9$ ) or BF ( $P=0.87$ ). Finally, no interaction effect was observed for either RF or BF ( $P=0.74$  &  $P=0.67$ , respectively; Table 6.2).

Table 6. 2. RMS% values for VL, RF, and BF during 10x6-second repeated sprints

	HEAT	IPC	IPC+HEAT	SHAM
VL RMS%	85 ± 3	78 ± 3	82 ± 3	84 ± 3
RF RMS%	80 ± 4	80 ± 2	78 ± 5	78 ± 4
BF RMS%	75 ± 4	76 ± 6	74 ± 4	75 ± 5

### 6.3.5 Skin Temperature

Skin temperature between preconditioning strategies was significantly different between HEATED and T-N preconditioning strategies (Figure 6.8).

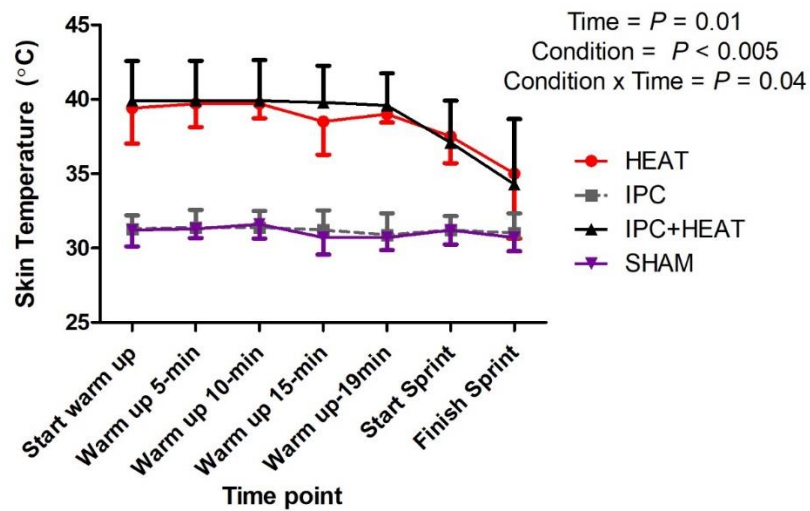


Figure 6. 8. Graphical representation of skin temperature during HEAT ( $38.4 \pm 1.9$  °C), IPC ( $31.2 \pm 1.1$  °C), IPC+HEAT ( $38.6 \pm 1.2$  °C) and SHAM ( $31.1 \pm 1$  °C).

## 6.4 Discussion

The primary aim of this study was to compare IPC and passive local muscle heating or a combination of both preconditioning strategies on RSA performance. Specifically, this study explored, for the first time, whether a 4x5-minute local IPC strategy in combination with local muscle heat maintenance following a standardised cycling warm-up could enhance RSA capacity. In contrast to our hypothesis, RSA performance was not enhanced by either IPC, local muscle heating, or a combination of both strategies when compared to a control (SHAM) condition.

### 6.4.1 Effects of IPC

In contrast to the original hypothesis, study findings showed no effect of IPC alone on power output during 10x6-second RSA cycling. Whilst early research suggested IPC did not alter RSA (5x6-second sprints off 24-second recovery) performance in team sport athletes (Gibson *et al.*, 2013), more recent work reported benefits of IPC on power output during a RSA cycling task (Patterson *et al.* 2015). In that study the peak power improvements occurred during the first 3 sprints *versus* SHAM suggesting IPC had a positive impact on anaerobic energy provision (Spencer *et al.*, 2005).

IPC has been proposed to elicit improvements in both anaerobic (Paradis-Deschênes *et al.*, 2016) and aerobic metabolism (de Groot *et al.*, 2010). Although this exercise task is highly anaerobic, the aerobic contribution to each sprint increases as RSA sets progress (Girard *et al.*, 2011). Therefore, aerobic capacity may become a limiting factor to RSA performance. Nevertheless, during a 6-second cycling sprint effort, anaerobic glycolysis and PCr degradation account for roughly 94% of total energy contribution (Spencer *et al.*, 2005). The largest magnitude of change in average power output *versus* SHAM was after IPC in the current study, however the typical error was greater than the observed change of 12 watts, thereby leading us to conclude any improvement observed was “unclear”. Previous work suggests IPC

meaningfully enhances performance by approximately 1.5% in well-trained individuals (Ferreira *et al.*, 2016), however the current enhancement *versus* SHAM was only 1.2%, possibly supporting the “unclear” inference. The trained cyclists in the current study were not track cyclists. Therefore, they were likely more aerobically trained *versus* other sprint-based athletes. Although further work is needed, IPC may become less effective in highly aerobic trained athletes (Hittinger *et al.*, 2015) compared to recreationally active individuals (de Groot *et al.*, 2010). Whether training status influences the effects of IPC on performance remains undetermined.

#### **6.4.2 Effects of increasing muscle temperature**

Higher muscle temperatures when commencing exercise did not result in enhanced power output during the RSA task, as originally hypothesized. A 1°C elevation in muscle temperature previously enhanced cycling peak power by almost 10% (Faulkner *et al.*, 2013a). Notably, the current study protocol employed a passive rest period of 14-minutes between the warm-up and RSA cycling, whereas the study of Faulkner *et al.* (Faulkner *et al.*, 2013a) employed a 30-minute passive rest period. This longer rest duration could explain the variance in findings between theirs, and the present data set. This is discussed in more detail below in *section 6.4.3*. In addition to elevating muscle temperature, it is possible that passively maintaining core temperature with blizzard jackets in cool conditions could enhance sprint running performance in team sport athletes (Kilduff *et al.*, 2013b). Importantly, studies using running as the primary sprinting modality, may be difficult to compare with cycling performance. Previous work documented greater sprint decrement scores (%) in cycling repeated sprint protocols *versus* running protocols (Girard *et al.*, 2011). Therefore, comparisons between the two exercise modes may be difficult to interpret due to inducing different fatigue responses. The current findings suggest that after 14-minutes of passive rest from a 19-minute incremental warm-up, consisting of sprint efforts, there is no need to passively maintain muscle temperature prior to performing 10x6-second

repeated sprints on a cycle ergometer. The warm-up protocol could be key to these findings and will be discussed in more detail below.

#### **6.4.3 Effects of combining interventions**

No benefit was observed after combining preconditioning strategies in the current study. The unclear change in performance between conditions may have partially been influenced by the choice of exercise bout. It is reported that intermittent tasks present lower inter-trial reliability when compared to fixed-end point, or constant load tasks (Hopkins, 2000). Regardless, the population used in the study were trained and showed good reliability before experimental trials commenced. Physiologically, it is unclear as to why no observed changes in either peak or average power were observed. It would be interesting to investigate whether a group of less-trained individuals would benefit from the measured interventions, based on previously discussed findings (Hittinger *et al.*, 2015).

To expand upon a point raised in the previous section, the warm-up in the current study aimed to induce a potentiation response (Racinais *et al.*, 2017) prior to the performance of RSA. Post-activation potentiation (PAP) is a mechanism consistently shown to benefit high-intensity performance in athletes (Scott *et al.*, 2016). Previous laboratory studies assessing the impact of either IPC or muscle heating interventions on anaerobic performance may have designed warm-up protocols that lacked sufficient exercise intensity (Cruz *et al.*, 2016), duration (Patterson *et al.*, 2015) or enforced a rest period beyond optimal (Faulkner *et al.*, 2013a). Previously, Faulkner *et al.* (Faulkner *et al.*, 2013a) investigated muscle temperature elevations on sprint performance and conducted a warm-up involving 5-minutes of steady state cycling followed by 5 x 10-second sprint efforts. The time between warm-up cessation and the sprint task was however 30-minutes, likely negating any warm-up potentiation effect (Rassier and Macintosh, 2000). Additionally, Patterson *et al.* (Patterson *et al.*, 2015) assessed the effect of IPC on RSA cycling and employed a 3-minute steady state cycling warm-up followed by 2 x maximal 6-

second efforts. The rest period in this study (5-minutes) was much more optimal (Sargeant and Dolan, 1987), however, the duration of the warm-up itself was likely insufficient to induce sufficient elevations in muscle temperature (Racinais *et al.*, 2017). The current study warm-up, lasting for 19-minutes in duration, was designed, based on how an athlete may try to optimise physiological readiness prior to competition (Bishop, 2003; Racinais *et al.*, 2017). Therefore, the current findings of “unclear” changes after IPC versus SHAM, may imply that a sufficient warm-up stimulus negates the beneficial response of IPC during predominantly anaerobic exercise tasks. Future research should aim to explore anaerobic tasks that are preceded by more appropriate warm-up procedures.

#### **6.4.4 Muscle characteristics to RSA**

Muscle oxygenation kinetics are likely important in RSA, due to the previously highlighted aerobic component to performance (Girard *et al.*, 2011). Although trends for enhanced muscle oxygenation responses were evident in heated conditions, “unclear” changes in power output were present. The impact of any changes at the muscular level were therefore likely insufficient to alter performance. Previously, it has been postulated that beneficial responses to IPC are induced via oxidative changes in the muscle. Based on the current evidence, during maximal repeated sprinting, any changes in muscle oxygenation are insufficient to exert meaningful responses on power production.

When discussing muscle activity, there was no impact of EMG RMS (%) between conditions. Patterson *et al.* (2015) reported a *possibly* higher median frequency in EMG after IPC *versus* SHAM. In the current study, RMS was analysed to control for the differences in muscle temperature between preconditioning strategies. Greater muscle temperatures during exercise can alter neurophysiological excitatory during contractions (For review, see Racinais *et al.*, 2017). The current data suggest no changes in the reduction in muscle activity as sprint efforts progressed.

#### **6.4.5 Limitations**

It is acknowledged that the largest limitation of this study is the sample size. The calculated typical error of the current task was 16 watts and 20 watts for average and peak power output, respectively but the small sample size likely made it difficult to accurately estimate changes in performance. However, it was previously shown that N=11 showed significant (9.6%) improvements in 30-second ‘all out’ sprint cycling with prior muscle heating, compared to a control (non-insulated) condition (Faulkner *et al.*, 2013a). Their group showed no significant change compared between the muscle heating condition and an (non-heated) insulated condition in that study (Faulkner *et al.*, 2013a). In the current study, all cyclists wore bib shorts during heated and T-N conditions, whilst elasticated tubigrips were used in T-N conditions to support and fix NIRS and EMG units. This may have acted as minor insulation in the T-N condition in the present study, but whether this explains the lack of significant differences between muscle temperature groups remains unknown. For sprint tasks, constant speed/power or duration tasks may provide better signal:noise ratios compared with intermittent protocols (Hopkins, 2000). Assessment of this type of performance task may allow more accurate determination of the impact of IPC or muscle heating on anaerobic performance. The major methodological limitation was muscle temperature was not directly measured in the current study. Nevertheless, validation of muscle heating methods was performed in a small number of participants to show muscle temperatures increased by 1°C at the start of sprinting, compared to thermo-neutral conditions. Additionally, this study documented skin temperature which also demonstrated clear differences between heated and T-N preconditioning strategies.

#### **6.4.6 Conclusion**

The current data suggest for the first time that RSA is unlikely further enhanced by implementing the “traditional” IPC dose, passively heating muscles to maintain post warm up temperature, or combining both strategies together when compared to a SHAM condition.

Moreover, it does not seem that IPC can enhance RSA cycling, regardless of whether implemented with thermoneutral or elevated muscle temperatures. This may provide greater clarity on the efficacy of IPC when integrated and compared with other popular preconditioning strategies.



# Chapter 7

## Thesis Synthesis

## 7.1 Major Findings

The major findings from this thesis are:

1. *When comparing the different IPC “doses” employed in the current thesis, the “traditional” 4x5-min IPC protocol may result induce preferential aerobic exercise performance.*
2. *Remote or local cuff placement mediates similar performance and blood flow responses to exercise.*
3. *Local “traditional” IPC enhances torque production before and after EIMD*
4. *Local “traditional” IPC does not impact cycling repeated sprint ability performance.*
5. *The combination of local “traditional” IPC and increasing muscle temperature does not enhance repeated sprint performance improvements.*

## 7.2 General discussion of findings

Many of the >40 investigations that are published on IPC and exercise at the time of writing this work, have employed different methodological approaches of how IPC is performed. Chapter 3 of this thesis highlights that the “traditional” 4x5 mins cycles may provide the most benefit to exercise performance. The occlusion of two limbs seems to be more effective than occluding just one limb and Remote or local cuff placement leads to similar performance outcomes. A linear dose-response to IPC is not present, with a greater number (8x5-minute) of cycles providing no additional benefit to a “traditional” 4x5-minute protocol. Importantly, a greater number of cycles was not harmful to performance which may be important finding when discussing ‘hyperconditioning’ with ischaemia. The importance of utilising “low-dose” ischaemic conditioning in clinical patients has previously been highlighted, following observations that 12x ischaemia-reperfusion cycles may induce deleterious collagen responses in the myocardium (Whittaker *et al.*, 1991; Whittaker and Przyklenk, 2014). The findings of the

current thesis highlight that employment of more (8x) ischaemia-reperfusion cycles is not damaging to exercise performance *versus* SHAM. Importantly, however, because no benefit is observed *versus* the “traditional” 4x5-minute IPC dose, no further cycles should be employed beyond four, prior to exercise. This is an important protocol finding for athletes contemplating the use of IPC prior to performance (Figure 7.1).

*What are the underlying mechanisms explaining exercise benefits of IPC?*

It is previously shown that IPC alters neuromuscular (Cruz *et al.*, 2015) and/or cardiovascular (Bailey *et al.*, 2012) function during or after exercise, respectively. The work in chapter 4 of this thesis assessed conduit artery responses during and following submaximal exercise. The findings of greater conduit artery dilation after local *versus* Remote IPC during submaximal handgrip exercise are novel. To date, study designs investigating vascular responses to IPC, either at rest (Enko *et al.*, 2011) or during exercise (Barbosa *et al.*, 2015) have not compared the location of cuff placement on vascular responses. Previous work investigating macrovascular responses during exercise has only been performed in a severe-domain intensity handgrip task to exhaustion (Barbosa *et al.*, 2015). The rationale for investigating submaximal exercise blood flow and vascular function responses was to try to establish a mechanistic link to further investigate how IPC can impact aerobic exercise performance (Salvador *et al.*, 2016).

Conduit artery responses to exercise were assessed, via ultrasound measurement, a technique deemed suitable for assessment of vascular function (Woodman *et al.*, 2001). During exercise, ultrasound measurement requires minimal limb movement to optimise artery image and enhance recording signal. Fixed angle, small muscle mass exercise tasks are therefore chosen to maximise the validity and reliability of measurements obtained. Nevertheless, the nature of small-muscle mass exercise e.g. handgrip exercise, is not representative of most athletic tasks. Only ‘whole-body’ exercise models can truly account for blood flow and vascular changes that

take place during hard exercise bouts, such as: 1) greater lower limb perfusion pressures (Joyner and Casey, 2015); 2) respiratory muscle blood flow demand and fatigue that competes with (or compromises) limb blood flow (Romer and Polkey, 2008); and 3) direct vascular responses such as metabo-reflex induced vasoconstriction (Romer and Polkey, 2008). Under whole-body exercise conditions, the conduit artery response to exercise would likely change. Development of methods to accurately measure blood flow changes during more intense ‘whole-body’ exercise tasks, will ultimately be needed to test the efficacy and validity of the findings in chapter 4 of this thesis.

No improvement in post-exercise vascular function was observed in chapter 4 between conditions. Previously, IPC improved post-exercise vascular function in the inactive (arm) limb *versus* a SHAM condition following 5-km running TT performance (Bailey *et al.*, 2012). The reduction in FMD in inactive limbs after strenuous exercise may be resultant from circulatory increases in active substances such as reactive oxygen species (ROS) (Taniyama and Griendling, 2003; Weseler and Bast, 2010). FMD assessments in chapter 4 of this thesis were performed on the exercising limb, unlike the study of Bailey *et al.* (Bailey *et al.*, 2012). It may be that active (exercising) limbs experience increases in arterial pressures, potentially inhibiting eNOS production via reduced sympathetic outflow (Green *et al.*, 2004). Inactive limbs however, may experience reductions in vasodilatory function, through other mechanisms, such as exposure to factors such as circulatory ROS. Although speculative, it may be postulated that IPC could offer “limb specific” systemic protection against circulatory ROS in inactive limbs, thereby improving FMD in inactive limbs.

*Can IPC prevent exercise-induced muscle damage in response to strenuous exercise?*

Chapter 5 expanded on the preliminary findings that IPC can positively influence muscle damage responses in humans (Franz *et al.*, 2017a) and also validated the rationale to use a 4x5-

minute “traditional” IPC dose prior to muscle damaging exercise. The findings of this chapter observed no changes in muscle torque recovery kinetics *versus* SHAM, a finding that contradicts previous work assessing the application of post EIMD intermittent occlusion bouts (Beaven *et al.*, 2012; Page *et al.*, 2017). Therefore, it remains unclear whether IPC specifically protects against muscle damage, or simply enhances exercise performance via a “delayed preconditioning” response, independent of EIMD.

The work of chapters 3 and 4 of this thesis directed and informed the implementation of what was perceived to be the “*preferred*” IPC protocol prior to exercise. Muscle damaging (eccentric) exercise is inherently different to a concentrically dominated performance task (Franz *et al.*, 2017b) and induces different contractile demands during an exercise bout (Asp *et al.*, 1998). Currently, there is no evidence to suggest that a more rigorous (greater amount of cycles / occlusion mass / repeated daily IPC) would induce different tissue-protective responses to the “traditional” dose of IPC that was employed. However, it could be speculated that the damaging nature of eccentric contractions could benefit from a greater priming (IPC) response and this is something that may deserve future research attention. Currently, the infancy of this research would infer that team sport, or competition setting athletes, that play on consecutive days, could benefit from this intervention. This is discussed in more depth in *section 7.3*.

*Does IPC lose potency when combined with other “beneficial” strategies?*

Both central and peripheral fatigue are limiting to repeated sprint exercise performance (Racinais *et al.*, 2007). Chapter 6 was only able to investigate a limited number of variables that potentially contribute to augmentation of repeated sprinting (Bishop *et al.*, 2011; Girard *et al.*, 2011). In an applied setting, the exposure to more external variables may further influence performance capability. One example of an introductory external variable when moving from the laboratory setting, to an applied setting may be the use of ergogenic aids (e.g. caffeine) prior

to exercise. Caffeine is a frequently used substance by athletes (Goldstein *et al.*, 2010) and the ability of caffeine to enhance performance is well documented (Goldstein *et al.*, 2010). It is not necessarily caffeine's ability to enhance performance, but instead, the physiological action of caffeine that is important to consider when applying IPC prior to exercise. IPC directly acts on the adenosine pathway (specifically acting on A<sub>1</sub> and A<sub>3</sub> receptor agonists) (Riksen *et al.*, 2004), whilst caffeine is a potent adenosine antagonist (Smits *et al.*, 1990, 1987), with a high affinity to adenosine (A<sub>1</sub> and A<sub>2A</sub>) binding sites (Huang *et al.*, 2005). This competitive binding action on adenosine receptors may explain abolished tissue protection following IPC when caffeine is administered (Riksen *et al.*, 2006). Additionally, other vasodilatory substances, capable of enhancing exercise capacity, such as dietary nitrates (Bailey *et al.*, 2010), have been shown to be ineffective when combined with or compared to traditional caffeine supplementation (Glaister *et al.*, 2015). Practically, *unless* athletes are caffeine intolerant or chose not to take caffeine for personal reasons, the efficacy of IPC to benefit the elite or competitive sports person may well be limited unless this work is undertaken to show otherwise. Advances in this research field are necessary for IPC to be proven efficacious in elite settings. This concept expands through all related chapters of this thesis, as ultimately, each chapter was designed to provide some inference into an area of exercise performance with the aim of eventually transitioning toward the elite performer.

Elite performers are also known to optimise warm-up strategies. It is possible, that warm-up protocols may influence the potency of IPC on subsequent anaerobic exercise tasks for reasons discussed in *section 6.4.3*. Whilst the warm-up was sufficient for chapter 3, the aim was not to induce a potentiation on the muscle, as this has only shown to benefit maximal (anaerobic) performance tasks (Racinais *et al.*, 2017). If the SHAM condition RSA task was afforded a potentiating response from warming up optimally in the work of chapter 6, this would again raise questions toward the efficacy of using IPC in elite sport, where warm-up procedures are

often optimised. Presently, several studies documenting benefits of IPC may have implemented sub-optimal warm-ups. Such a simple methodological consideration may be of vital importance in determining the efficacy of IPC in elite sport.

#### *Does exercise mode matter?*

Whilst physiological fatigue mechanisms were not directly measured, IPC has been suggested to enhance central motor drive (Cruz *et al.*, 2015), via a IPC-induced blunting of group III and IV afferent feedback (Crisafulli *et al.*, 2011). Group III and IV afferents respond to exercise-induced metabolite accumulation in the interstitial space (Burnley and Jones, 2018), and their activation/firing are thought to limit capacity during severe, or extreme domain exercise (Amann *et al.*, 2015). The action of IPC on these afferents has been widely discussed (Angius *et al.*, 2017; Cruz *et al.*, 2017) and if enhancement of central motor drive is key to the improvement of supramaximal performance, an exercise task that does not allow recovery (even incomplete recovery) between efforts, may better elucidate mechanisms that contribute to enhancements in supramaximal exercise capacity. It may therefore be interesting to determine the impact of IPC on fixed-end point or constant duration extreme-domain exercise bouts that do not allow (partial) recovery throughout the effort.

### 7.3 Practical implications for athletes

Presently, it appears that employment of a 4x5-minute “traditional” protocol on the working musculature elicits the greatest physiological responses during exercise, as determined from the work in this thesis.

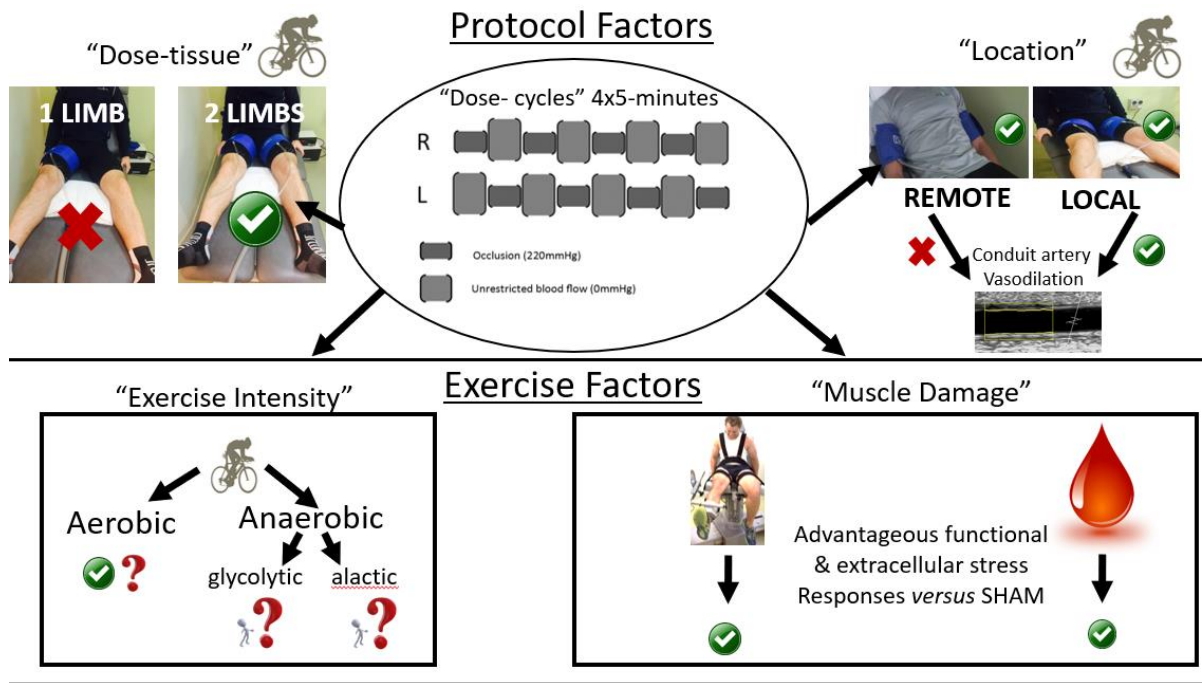


Figure 7. 1. A schematic representation of the optimal protocol for athletes to employ IPC as a pre-exercise strategy based on the current research evidence and the findings from this thesis.

A “traditional” IPC protocol applied locally to the working limbs may provide benefit to endurance TT performance in trained cyclists (figure 7.1), however testing IPC in elite athletes, alongside the consumption or use of other pre-race (nutritional) ergogenic strategies (such as caffeine) is needed to determine whether beneficial findings of IPC are ecologically valid. Importantly, in addition to the wealth of research on the potential impact of IPC to enhance exercise performance, this thesis contributed to the field of muscle damage, with the suggestion that IPC may also prevent a decline in exercise performance typically experienced from EIMD. Indeed, the promising findings in chapter 5 suggests that development of future studies assessing the EIMD responses to IPC, in more applied sporting settings, may be warranted. Presently, there is no sport specific data to suggest IPC could benefit force production or exercise capacity during competition settings. Currently these data do not present sufficient evidence for IPC to be used by athletes. When postulating the long-term application of using IPC to prevent



deleterious effects of muscle damage, tournament settings could be an ideal target. Tournament settings are often characterized by playing numerous games or competing in several events in a short period of time. The caveat to condensed sporting schedules is an inability to recovery physically between games/competition, especially if competition is comprised of numerous eccentric or deceleration loading tasks. The work contained in this thesis provides rationale to expand and develop understanding of both the muscle (mechanical) and systemic (biomarker) responses to IPC when more tournament sports-specific tasks are performed. Many team sport events often contain numerous deceleration or eccentrically loaded movements (Russell *et al.*, 2016), sometimes even repetitive player contacts (Singh *et al.*, 2011) that can lead to muscle damage, alongside reductions in muscle function in subsequent days that follow competition (Ascensão *et al.*, 2008).

#### 7.4 Recommendations for future work

1. Is there a ‘minimal effective’ dose of IPC?

Coaches and athletes are often time-restricted prior to competitive events, therefore, any finding of a ‘minimal effective dose’, would be highly impactful when aiming to employ IPC in an applied setting. The 3x5-minute IPC protocol, as first assessed in the seminal IPC and exercise study (de Groot *et al.*, 2010) has shown to be effective in a range of tasks. Additionally, enhanced recovery from exercise was observed following 2 x 3-minute occlusion cycles (Beaven *et al.*, 2012). A direct comparison of shorter protocols when compared to the 4x5-minute local IPC protocol used throughout this thesis may be warranted to establish whether a more time-effective IPC strategy exists.

2. Is there a mechanistic response to delayed IPC?

This thesis did not focus on the application of delayed (approx. 24 hours prior to exercise) IPC, which also may provide a time benefit to the competing athlete (Seeger *et al.*, 2016). The impact

and effectiveness of delayed IPC remains unclear and has only been investigated using performance tasks (Seeger *et al.*, 2016). New mechanistic work has recently been published, showing red blood cell deformability can potentially establish IPC responders (Tomschi *et al.*, 2018). A technique such as this could be utilised within this type of study. Additionally, investigation into other physiological mechanisms, such as vascular responses during exercise may provide better insight into whether delayed IPC is a strategy worth pursuing for the athlete. This may help to establish whether, as previously proposed (Seeger *et al.*, 2016), participants may be “responders” to IPC, regardless of timing.

### 3. Can IPC alter vascular responses to whole body exercise?

Increasing the amount of working musculature, alters the limb blood flow demand of skeletal muscle, as previously discussed in this chapter. Additionally, more intense ‘whole-body’ exercise may alter the vasodilatory responses to an exercise task (Romer and Polkey, 2008). To truly determine the impact IPC may have during intense exercise, conduit artery measurement should ideally be established during a whole-body exercise task, across a range of intensities. The replication of a similar study design to chapter 4, involving higher intensity, lower limb bilateral isometric contractions may allow for accurate ultrasound measurement and provide a greater understanding and/or validation of the current thesis findings relating to conduit artery function.

### 4. Can IPC enhance simulated team sport performance in athletes experiencing eccentric-exercise induced muscle damage?

The work in this thesis currently demonstrates that IPC enhances maximal torque production before and after EIMD. To better apply these findings to athletes, further investigation of a controlled exercise task, representative of team sport competition demands, is needed. Using a similar design to chapter 5, a simulated soccer protocol could be employed using a non-

motorized treadmill (Aldous *et al.*, 2014). The protocol could be performed on a baseline (pre-ECC) visit and then at 24- and 72-hrs after eccentric exercise (EIMD) has occurred. Blood markers could also be taken to further investigate systemic extracellular stress responses to exercise, expanding on work performed in the current thesis. This research would develop a better practical understanding of whether IPC could meaningfully benefit team sport athletes that experience muscle damage in tournament-style settings.

#### 5. Can exercise preconditioning reduce the impact of IPC on anaerobic performance?

If the eventual aim of IPC research is to inform athletic practice, future studies should aim to replicate applied practice more accurately. A simple example of this, is by using more appropriate warm-ups prior to laboratory tasks. Not only can exercise alone be a preconditioning stimulus in a similar manner to IPC (Crisafulli *et al.*, 2007), performing a suitable warm-up can induce a potentiating response during high-intensity (anaerobic) exercise performance. Currently, most studies showing benefit to IPC in anaerobic tasks, have failed to employ “optimal” warm-up protocols. Whether IPC would augment anaerobic performance if exercise was potentiated naturally (via an optimised warm-up) remains unclear. This research may again lead to better understanding of how effective IPC may be in elite settings.

#### 6. Would a combined ‘warm-up’ strategy provide larger performance benefits on constant load or constant duration anaerobic tasks?

Elevated muscle temperature can be ergogenic to anaerobic, power-based exercise tasks (Faulkner *et al.*, 2013b) if a sufficient rest time is undertaken between the warm-up and exercise bout. Based on the suggestion that IPC could potentiate anaerobic metabolism (Paradis-Deschênes *et al.*, 2016) a similar study design to chapter 6, prior to an “all out” one-off maximal task may provide greater clarification as to whether this practical preconditioning model can enhance anaerobic performance. Additionally, it is plausible that longer duration maximal

efforts may be a more appropriate exercise task to further investigate central motor drive responses to IPC. Importantly, many of the studies on IPC to date have relatively small sample sizes. It may therefore be more appropriate to assess the impacts on sprint performance by using tasks shown to reduce the noise of a test (improving the signal:noise ratio) (Hopkins, 2000).

7. What are the vascular and performance responses to IPC and caffeine in trained individuals?

Currently, IPC has not been implemented with “elites” in applied settings. If the eventual goal is to develop this strategy in elite performers, it needs to be first tested in ecologically valid settings, which may include the use of ergogenic aids such as caffeine. The ergogenic response to caffeine is widely published (Goldstein *et al.*, 2010) and it is highly popular substance among athletes, since its re-instatement by the world anti-doping agency (WADA). For IPC to be considered worthwhile in athletes that consume caffeine, a range of performance tasks that are undertaken with either caffeine, *versus* or in combination with IPC, should be performed. A replication of a model similar to that employed by Bailey *et al.* (2012) whereby vascular function assessments are performed pre- and post-severe domain exercise could be insightful at both a performance and mechanistic level. The performance of an exercise task that elicits (near to)  $\dot{V}O_{2max}$  may be optimal for this type of study. This advance in research direction is vital for IPC to (if ever) be considered on the elite stage.

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# Appendices

## Appendix 1

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## Appendix 2

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## Appendix 3

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