THE INFLUENCE OF COLD WATER IMMERSION ON LIMB BLOOD FLOW AND THERMOREGULATORY RESPONSES TO EXERCISE

Chris Mawhinney

A thesis submitted in partial fulfillment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

May 2016

Abstract

The accumulated stresses of training and competition may temporarily cause impairments in an athlete's physiological and muscular function, leading to suboptimal performance levels. Cold-water immersion (CWI) has become a widely used post-exercise recovery method to accelerate the recovery process by purportedly reducing the symptoms associated with exercise-induced muscle damage (EIMD). However, the underlying physiological mechanisms, which mediate the effects of CWI, are not well understood. Therefore, the aim of this thesis was to investigate the influence of cold-water immersion (CWI) on limb blood flow and thermoregulatory responses following different modes of exercise.

In study 1 (Chapter 4), the reliability of Doppler ultrasound in the assessment of superficial femoral artery blood flow (FABF) was examined under resting conditions. A Doppler ultrasound scan of the superficial femoral artery was measured on eight recreationally active male participants; twice on the same day separated by 5-min (within-day), and on a separate day (between-days). The coefficient of variation (CV) for mean blood flow (MBF) was ~16 % and ~20 % for within and between-days, respectively. A relatively small standard error of measurement (SEM) was found both within day, 13.30 $mL \cdot min^{-1}$ (95% CI, -14.79 to 38.40 $mL \cdot min^{-1}$) and between-day, 17.75 $mL \cdot min^{-1}$ (95% CI, -40.12 to 30.88 $mL \cdot min^{-1}$) for MBF differences. These findings suggest duplex Doppler ultrasound is a reliable method to collect measurements of FABF under resting conditions.

The purpose of study 2 and 3 was to determine the influence of different degrees of water immersion cooling on FABF and cutaneous blood flow (CBF) and thermoregulatory responses after endurance (Chapter 5) and resistance (Chapter 6) exercise, respectively. Participants completed a prescribed endurance of resistance exercise protocol prior to immersion into 8 ºC (cold) or 22 ºC (cool) water to the iliac crest or rested non-immersion (CON) in a randomized order. Limb blood flow and thermoregulatory responses were measured before and up to 30-min after immersion. In both studies, thigh skin temperature (Tsk_{thigh}) ($P < 0.001$) and muscle temperature (T_{muscle}) ($P < 0.01$) were lowest in the 8 ºC trial compared with 22 ºC and control trials. However, femoral artery conductance (FVC) was similar after immersion in both cooling conditions and was reduced (~50-55 %) compared with the CON condition 30-min after immersion ($P < 0.01$). Similarly, there was a greater thigh ($P < 0.01$) and calf ($P < 0.05$) cutaneous vasoconstriction during and after immersion in both cooling conditions relative to CON with no differences noted between 8 and 22 ºC immersion. Together, these findings suggest that colder water temperatures may be more effective in the treatment of EIMD and injury after both endurance and resistance exercise, respectively, due to greater reductions in T_{muscle} and not limb blood flow *per se*.

The aim of study 4 (Chapter 7) was to compare the influence of CWI and whole body cryotherapy (WBC) on FABF and CBF and thermoregulatory responses after endurance exercise. On separate days, participants completed a continuous cycle

ergometer protocol before being immersed semi-reclined into 8 ºC water to the iliac crest for 10 min (CWI), or exposed to 2.5 min (30 s -60 ºC, 2 min -110 ºC) WBC in a specialized cryotherapy chamber, in a randomized order. Limb blood flow and thermoregulatory responses were measured before and up to 40-min after immersion Reductions in Tsk_{thigh} ($P < 0.001$) and T_{muscle} ($P < 0.001$) were larger in CWI during recovery. Similarly, decreases in FVC were greater (~45-50 %) in the CWI condition throughout the recovery period ($P < 0.05$). There was also a greater skin vasoconstriction observed in CWI at the thigh ($P < 0.001$) and calf ($P < 0.001$) throughout the post-cooling recovery period. These results demonstrate that CWI may be a better recovery strategy compared with WBC due greater reductions in both T_{muscle} and limb blood flow.

This thesis provides a novel insight into the influence of different degrees of water immersion cooling, as well as WBC, on limb blood flow and thermoregulatory responses after different modes of exercise. These findings provide practical application for athletes and an important insight into the possible mechanisms responsible for CWI in alleviating inflammation in sport and athletic contexts.

ACKNOWLEDGEMENTS

I would like to thank Professor Warren Gregson (director of studies) for his guidance, thoughtful comments, patience, understanding and continued support throughout the completion of this doctorate. I will forever be indebted for the opportunity to undertake this thesis and for my academic development; I hope to continue to work together in the near future.

I am also indebted to Dr Helen Jones (2nd supervisor), Professor Danny Green (3rd supervisor) and Dr David Low for their insightful comments, support and academic discussions, which have helped shape this thesis.

This thesis would not have been possible without the efforts of the participants who volunteered to take part in each study, your time and effort was really appreciated.

My gratitude and sincere thanks to my fellow doctoral students Chang Hwa Joo and Rob Allan for their assistance during testing and to the technical staff, especially George Savage, for helping fill, empty, clean and chlorinate the water baths. You can wash your hands of me now…pun intended!!

I would like to acknowledge U.K. Sport for the funding they provided to enable this thesis to be undertaken, Air Products Ltd for the use of their whole body cryotherapy chamber and ECB Cold Spa Ltd for their donation of the water tanks and cooling system. I hope the findings of this thesis are of interest to you all.

Finally, I would like to thank my mother for her continued support and my girlfriend Saiphon for her patience and encouragement.

I dedicate this thesis to my late father, Derek Mawhinney.

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 8

CHAPTER 9

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
AU	Arbitrary Units
ANS	Autonomic Nervous System
BP	Blood Pressure
Ca+	Calcium Ions
Tsk_{calf}	Calf Skin Temperature
$\dot{Q}$	Cardiac Output
CBV	Central Blood Volume
CNS	Central Nervous System
CVP	Central Venous Pressure
Δ	Change
Tsk_{chest}	Chest Skin Temperature
CV	Coefficient of Variation
CWI	Cold Water Immersion
CON	Condition
CI	Confidence Interval
CMJ	Counter Movement Jump
CK	Creatine Kinase
CBF	Cutaneous Blood Flow
CVC	Cutaneous Vascular Conductance
DOMS	Delayed Onset Muscle Soreness
D	Diameter
ES	Effect Size
E-C Coupling	Excitation-Contraction Coupling
EIMD	Exercise Induced Muscle Damage
FVC	Femoral Artery Vascular Conductance
$Tsk_{forearm}$	Forearm Skin Temperature
GLM	General Linear Model
HR	Heart Rate
H+	Hydrogen Ions
P_i	Inorganic Phosphate
La	Lactate
LIST	Loughborough Intermittent Shuttle Test
MRI	Magnetic Resonance Imaging
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
MVC	Maximal Voluntary Contraction
MVIC	Maximal Voluntary Isometric Contraction
MAP	Mean Arterial Pressure
MBF	Mean Blood Flow
MBV	Mean Blood Velocity
Tsk_{mean}	Mean Skin Temperature
T_{muscle}	Muscle Temperature
Mg	Myoglobin
NIRS	Near Infrared Spectroscopy
$\dot{V}O_{2peak}$	Peak Oxygen Uptake
PET	Positron Emission Tomography
K+	Potassium ions

PO/AH	Preoptic Anterior Hypothalamus
ROM	Range of Motion
RPE	Ratings of Perceived Exertion
ROS	Reactive Oxygen Species
T_{rec}	Rectal Temperature
RM	Repetition Maximum
SEM	Standard Error of Measurement
SV	Stroke Volume
FABF	Superficial Femoral Artery Blood Flow
Tsk_{thigh}	Thigh Skin Temperature
TPR	Total Peripheral Resistance
TMS	Transcranial Magnetic Stimulation
VOP	Venous Occlusion Plethysmography
WBC	Whole Body Cryotherapy
Yo-Yo	Yo-Yo Intermittent Recovery Test
Xe^{133}	133Xenon

LIST OF FIGURES

CHAPTER 6.

CHAPTER 7.

LIST OF TABLES

CHAPTER 1
GENERAL INTRODUCTION

1.1. BACKGROUND

The accumulated stresses of training and competition may temporarily cause impairments in an athlete's physiological and muscular function, leading to suboptimal performance levels (Peiffer *et al*., 2009b; Schniepp *et al*., 2002). This decrement in performance capability may be associated with high-intensity exercise, which causes metabolic disturbances and impairs performance in the acute period after exercise, i.e. minutes and hours (Barnett, 2006; Westerblad, Allen & Lannergreen, 2002). Alternatively, the mechanical stress associated with exercise, which involves a high number of eccentric contractions, may cause exercise-induced muscle damage (EIMD) and lead to delayed onset muscle soreness (DOMS) in the days after exercise (Barnett, 2006; Connolly, Sayers & McHugh, 2003). It is therefore apparent, that in order to maximize athletic performance, a balance is required between the accumulated stresses from training and/or competition and recovery.

Recovery strategies have become commonplace in athlete training regimes in an attempt to speed up the recovery process. One such strategy, cold-water immersion (CWI), has become a popular and widely used method by both recreational and elite athletes alike (McGorm *et al*., 2015). Cryotherapy, often in the form of ice packs, has traditionally been prescribed in immediate treatment and management of soft tissue injury to attenuate the symptoms inherent in the inflammatory response. Since inflammation is also integral in the development of EIMD (Smith, 1991), CWI has increasingly been applied to exercised limbs to augment recovery; typically using temperatures in the range of 10 to 15ºC and durations of 10-15 min (McGorm *et al*., 2015). To date, the effects of CWI has been shown to have an inconsistent effect on

performance recovery and markers of muscle damage, possibly reflecting the different modes of exercise and types of cooling, i.e. temperature assigned.

The physiological mechanisms underpinning the use of CWI remain unclear. The benefits of CWI are proposed to be partly related to temperature-induced reductions in microvascular blood flow around an injured muscle (Gregson *et al*., 2011), which reduces oedema and inflammatory events (Gregson *et al*., 2011; Lee *et al*., 2005; Thorlacius *et al*., 1998). Despite the direct measurement of muscle blood flow not currently being possible, indirect estimates have been attained after post-exercise CWI using venous occlusion plethysmography (VOP; Vaile *et al*., 2011) and near infrared spectroscopy (NIRS) (Ihsan *et al*., 2013; Roberts *et al*., 2015a) techniques, respectively. Unfortunately, these methods are limited in the measurement of muscle blood flow, since VOP does not distinguish between muscle and skin blood flows (Gregson *et al*., 2011; Vaile *et al*., 2011) and the NIRS method only measures changes in muscle blood volume and not flow *per se* (Koga *et al*., 2015). Furthermore, the NIRS method may be confounded when changes in skin blood flow arise, such as with exercise and cooling (Davis *et al*., 2006). Recently, simultaneous measurements of femoral artery blood flow (FABF) and cutaneous blood flow (CBF) have provided an indirect estimate of muscle blood flow in the lower limbs in response to cold (8 ^{o}C) and cool (22 ^{o}C) water immersion (Gregson *et al*., 2011). This technique has advantages over VOP and NIRS assessments of muscle blood flow since it provides absolute blood flow measures and has an improved temporal resolution compared with VOP (30 Hz compared with 1 measure every 5-10 *s* with VOP) (Gregson *et al*., 2011). Under resting conditions, it was reported that both water temperatures promoted similar reductions in limb blood flow but 8 ^{o}C water resulted

in a higher blood flow to the skin. This suggests that colder water temperatures may induce greater reductions in muscle blood flow at rest (i.e. not after exercise) and be more effective in the treatment of EIMD and injury. Currently no attempt has been made to compare the effects of different degrees of water-cooling on limb blood flow responses after exercise. This is important to establish because CWI is often applied to exercised limbs as opposed to non-exercise (resting) conditions. Furthermore, it remains to be elucidated whether contrasting modes of exercise, e.g. endurance and resistance exercise, which may impose a different metabolic, thermal and hemodynamic strain on the body, can modify the limb blood flow response to CWI. Such information has important implications for treatment guidelines because the application of CWI frequently occurs after exercise when core body and local limb temperatures are elevated.

Recently, whole body cryotherapy (WBC), which exposes the limbs to extreme cold air temperatures (-110 °C to -140 °C) for a short duration (1-4 min), has become a popular post-exercise recovery method. To date, only one study has attempted to examine the limb blood flow response to WBC using the NIRS technique and under resting conditions (Selfe *et al*., 2014). Whilst the study determined that changes in muscle blood volume may be reduced after WBC exposure, a comparison with a CWI intervention was not included. Therefore, it remains to be elucidated, which method (CWI or WBC) is the optimal cryotherapy recovery strategy to be employed after exercise. The beneficial effects of WBC are also partly related to temperature-induced reductions in microvascular blood flow (White & Wells, 2013). Consequently, different modes of cryotherapy, i.e. air and water, which have different thermal gradients and durations of exposure, may lead to different rates of tissue temperature

change (White & Wells, 2013) and modulations in limb blood flow (Barcroft & Edholm, 1943). This is an important consideration for sports practitioners and athletes who wish to optimize athletic recovery after training and competition to improve performance.

1.2. AIMS AND OBJECTIVES

The aim of this thesis was to examine the influence of different degrees of lower body water immersion cooling on limb blood flow and thermoregulatory responses after different modes of exercise. Moreover, these physiological responses to CWI will be compared against WBC after exercise.

The aims of this thesis will be achieved by the following objectives:

Objectives

I. To examine the reliability (measurement error) of Doppler ultrasound as a measure of femoral artery blood flow under resting conditions.

II. To determine the influence of different degrees of cold-water immersion on limb blood flow and thermoregulatory responses after endurance exercise.

III. To determine the influence of different degrees of cold-water immersion on limb blood flow and thermoregulatory responses after resistance exercise.

IV. To compare the limb blood flow and thermoregulatory responses to cold-water immersion and whole body cryotherapy after endurance exercise.

CHAPTER 2

REVIEW OF LITERATURE

2.1. LITERATURE SEARCH

A computerized literature search was conducted using Medline (PubMed), SportDiscus and Google Scholar for peer reviewed journals. The following key phrases and their combinations were used: cold water immersion, whole body cryotherapy, ice bath, exercise recovery, recovery strategy, recovery modality, central fatigue, peripheral fatigue and exercise induced muscle damage. Reference lists were also examined for identification of any further eligible studies. In reviewing the effect of CWI on performance after high intensity exercise and muscle damage after eccentric exercise (see Table 2.6.1 and 2.6.2), only studies, which used passive recovery i.e., resting in ambient air or thermoneutral water, were included. Studies, which used active recovery, i.e. exercise as the control, were excluded.

2.2. EXERCISE RECOVERY

The accumulated stresses of training and competition may temporarily impair an athlete's physiological and muscular function, leading to suboptimal performance levels (Crowe *et al*., 2007; Peiffer *et al*., 2009; Schniepp *et al*., 2002). The decrement in performance may be emphasized when inadequate recovery periods are provided before the next training session or competitive fixture, particularly if a series of multiple competitive fixtures and/or high volume training sessions are completed over an acute phase, i.e. tournaments. Conversely, the more successful a team or an individual becomes, a greater number of competitive fixtures and/or training sessions are encountered, leading to an increased chance of overreaching or overtraining syndrome (Budgett, 1998). Therefore, it is important to find an appropriate balance between accumulated stresses and recovery to maximize athletic performance (Barnett, 2006). This has led to recovery modalities becoming commonplace in many

individual and team based sports training regimens to reduce associated physiological and functional deficits and speed up the recovery process.

2.3. FATIGUE

A bout of exercise elicits stress on the body, which may cause physiological impairments that lead to fatigue; an exercise-induced reduction in the ability of the muscle to generate the required or anticipated force or power during exercise (Bigland-Ritchie & Woods, 1984; Søgaard *et al*., 2006, Taylor *et al*., 2016). Fatigue is specific to the task being performed; hence there is no global mechanism, which is directly responsible for it's development (Enoka & Duchateau, 2008) Rather, reduced force production involves a combination of neural, metabolic and mechanical processes, with a failure in any factor contributing to fatigue (Allen *et al*., 1992).

2.3.1. Central fatigue

The central factors in fatigue are related to the central nervous system (CNS) activation required for voluntary movement. Central factors include the stimulation of the motor cortex in the brain, which leads to innervation of α-motor neurons in the spine and the innervation of the muscle fibers that power activity (Coyle, 2000). This is achieved via action potentials that travel along motor nerves and excite the muscle via neuromuscular junctions (Allen *et al*., 1992). The neural drive to muscle determines if, when and to what degree muscle fibers are activated, contributing to a decline in force and power and compromised performance (Taylor *et al*., 2016).

The central fatigue hypothesis proposed by Newsholme *et al* (1987), implicated exercise-induced changes in the concentration of the brain neurotransmitter serotonin,

to negatively impact on arousal, lethargy, sleepiness and mood (Newsholme, Acworth & Blomstrand, 1987; Taylor *et al*., 2016). It was suggested that this mechanism could influence perception of effort and associated fatigue (Newsholme, Acworth & Blomstrand, 1987; Taylor *et al*., 2016). However, manipulation of the serotonin neurotransmitter system has shown this mechanism to have an inconsistent effect on performance (Roelands & Meeusen, 2010) and is therefore not thought to be a key factor in central fatigue (Taylor *et al*., 2016). A similar equivocal effect on performance during exercise has also been reported for dopamine and noradrenaline neurotransmitters under normal ambient conditions (Taylor *et al*., 2016). In contrast, under high ambient temperatures (> 30 °C), noradrenaline and dopamine have been shown to have a clearer effect on fatigue and performance (perceived exertion, pacing strategy and core temperature), by modulating thermoregulatory responses via neurotransmitter hypothalamic pathways (Taylor *et al*., 2016).

Central fatigue has also been studied using maximal voluntary isometric contractions (MVC), since it tests the entire motor pathway (Taylor & Gandevia, 2008). Stimulation of the motor nerve and transcranial magnetic stimulation (TMS) of the motor cortex have been shown to elicit superimposed twitches and an increment in muscle force during a maximal effort contraction (Gandevia *et al*., 1996; Todd, Taylor & Gandevia, 2003). The observation of superimposed twitches from motor nerve and cortical stimulation indicates some motor units are either not recruited and/or not rapidly enough. This infers some central fatigue and loss of muscle force is not just located proximal to the site of motor axon stimulation, but also higher at the supraspinal level (Taylor & Gandevia, 2008).

Submaximal repetitive contractions leads to muscle fibers gradually becoming fatigued via increased voluntary effort required to recruit more motor units and/or increased firing rates to a point where maximal effort is reached (Taylor & Gandevia, 2008). This has been established using interspersed measurement of MVC during a submaximal task, evoking superimposed twitches via stimulation of the motor nerve (Smith *et al*., 2007). In addition, during submaximal contractions, TMS has demonstrated that there is a progressive descending cortical drive, indicating incremental increases in supraspinal fatigue (Smith *et al*., 2007; Søgaard *et al*., 2006). In contrast to maximal effort tasks, these observations suggest that central fatigue does not require a high level of cortical input, or recruitment of a large proportion of the motoneuron pool (Taylor & Gandevia, 2008). Since central fatigue is present despite most of the motor pathway not being engaged, it suggests that afferent inputs (particularly type iii and iv muscle afferents) contribute to reduce voluntary activation during prolonged activity (Taylor & Gandevia, 2008). These findings signify the development of central fatigue alongside peripheral fatigue (Smith *et al*., 2007; Taylor & Gandevia, 2008) during submaximal/prolonged activity.

2.3.2. Peripheral fatigue

Peripheral factors implicated in muscle fatigue are numerous, however, primary mechanisms include the accumulation of body heat, metabolic inhibition of the muscles contractile process, excitation-contraction coupling (E-C coupling) failure and fuel substrate depletion (Ament & Verkerke, 2009; Kent-Braun, 1999). High-intensity exercise at maximal or near maximal exercise intensities (e.g. sprinting, 400 m) also cause a rapid decline in muscle function via accumulation of intramuscular metabolites (Kent-Braun, 1999; Westerblad, Allen & Lännergren, 2002). Whilst the

particular metabolite, which plays the major role in high-intensity exercise fatigue, is yet been ascertained, current theories have moved away from the traditional increase in hydrogen ions (H+), acidosis and low pH causative approach to implicate inorganic phosphate (P_i) and potassium (K+) as key contributors (Westerblad, Allen & Lännergren, 2002). Indeed, mammalian muscle studies have shown low pH to have little effect on muscle contraction, whereas increased P_i levels interfere with the muscle contractile process (Dahlstedt & Westerblad, 2001; Fryer *et al*., 1995; Westerblad, Allen & Lännergren, 2002). The accumulation of interstitial potassium has been attributed to cause fatigue during intense exercise due to impairment of membrane excitability (blocking of t-tubule action potential and impaired E-C coupling (Nielsen *et al*., 2004; Fitts, 1994).

During prolonged high-intensity exercise (e.g. marathon running, triathlon), dehydration and the depletion of carbohydrate stores become of increasing importance in producing fatigue (Westerblad, Allen & Lännergren, 2002). Longer lasting types of exercise lead to an accumulation of body heat, increased sweating and dehydration via loss of water (Cleary, Sitler & Kendrick, 2006). This can affect muscle cell function and interfere with actin-myosin cross-bridge formation (Cleary, Sitler & Kendrick, 2006; Hargreaves & Febbraio, 1998) causing fatigue and a reduction in exercise performance. The predominant reliance of carbohydrate metabolism, via utilization of the oxidative glycolytic system, may also lead to fatigue via related decreases in local muscle/liver glycogen depletion (Karlsson, 1979). A direct cause and effect relationship between glycogen depletion and impaired muscle function remains to be established (Ørtenblad, Westerblad & Nielsen, 2013). However, it is well documented that a close relationship exists between muscle

glycogen content and fatigue resistance (Hargreaves, McConell & Proietto 1995; Ørtenblad, Westerblad & Nielsen, 2013), with low glycogen levels generally considered to compromise the rate of adenosine triphosphate (ATP) regeneration (Jensen & Richter, 2012; Ørtenblad, Westerblad & Nielsen, 2013). Furthermore, it has been proposed that a glycogen-dependent role may be present in the E-C coupling failure, which leads to fatigue, with a pool of intra-myofibrillar glycogen thought to counteract impaired sarcoplasmic reticulum calcium ion (Ca+) release (Østenblad, Westerblad & Nielsen, 2013).

2.4. EXERCISE-INDUCED MUSCLE DAMAGE

Different types of intense exercise cause varied perturbations in energy substrate depletion, thermal body status, mechanical muscle damage, oxidative stress, inflammation and nervous system fatigue (Leeder *et al*., 2012). For example, eccentric exercise may cause a relatively large mechanical stress with a low metabolic cost (Lastayo *et al*., 1999; Leeder *et al*., 2012) compared with intermittent sprint exercise, which has both a large mechanical stress and high metabolic cost (Leeder *et al*., 2012; Thompson, Nicholas & Williams, 1999). Consequently, a different time course of recovery from fatigue may be observed between different exercise stressors (Leeder *et al*., 2012). A longer duration of recovery may result from types of exercise, which cause exercise-induced muscle damage (EIMD), particularly during exercise that is unaccustomed (Howatson *et al*., 2008), has a predominance of muscle stretching (Keeton & Binder-Macleod, 2006), and/or a greater than normal intensity or duration (Leeder *et al*., 2012; Tee, Bosch & Lambert, 2007). EIMD typically leads to oedema, swelling and delayed-onset muscle soreness (DOMS), the sensation of tenderness, pain or discomfort, which may restrict movement and last for several days

(Connolly, Sayers & McHugh, 2003; Howatson *et al*., 2008; Warren *et al*., 2001). Importantly, EIMD can lead to a rise in passive muscle tension and a temporary loss of muscle force production (Howatson *et al*., 2008; Morgan & Allen, 1999; Warren *et al*., 2001) having a profound affect on the ability to perform subsequent bouts of exercise (Howatson *et al*., 2008).

The manifestation of oedema and swelling, associated with muscle damaging exercise, increases mechanical stress on the cellular structures, compresses capillaries and impairs O_2 delivery and waste removal leading to DOMS (White & Wells, 2013; Wilcock, Conin & Hing, 2006). The accompanying change in cell permeability may also lead to an increased intracellular accumulation of Ca+ (Gissel, 2005), which activates proteases (protein catabolism enzymes) and inflammatory signaling cell processes (White & Wells, 2013). Whilst the inflammatory response promotes muscle fiber restoration, it includes infiltration of neutrophils and macrophages to scavenge cellular debris, which can cause secondary enzymatic injury and further tissue damage and/or necrosis in healthy fibers through lysosome mechanisms (degradative enzymes) (Merrick, 2002). Secondary ischemic injury leads to further tissue damage through a lack of oxygen, which brings about additional oedema and further cell death (Merrick, 2002) and compounds the soreness and muscle force generating capacity in the hours and days after exercise (White & Wells, 2013). The restriction of secondary injury via reduction in the rate of chemical reactions and/or metabolic demand is the main basis of cryotherapy intervention methods to speed up the recovery process (Merrick, 1999; Figure 2.4.1).

2.4.1. Eccentric contractions

It is well documented that unaccustomed exercise per se and/or exercise, which involves a high number of eccentric contractions, i.e. resistance, plyometric exercise and/or multiple deceleration and turning exercises, imparts a high degree of mechanical stress on the active muscle/s (Armstrong *et al*., 1991; Fridén & Lieber, 1992; Morgan & Allen, 1999; Warren *et al*., 2001). The presence of disrupted sarcomeres in myofibrils and damage to the excitation-contraction coupling system are two prominent signs of muscle damage after eccentric exercise (Proske & Morgan, 2001). It remains unknown, which of these two signs is the primary event after eccentric EIMD. However, in brief, repeated eccentric lengthening of muscle fibres requires cross bridges to produce force whilst lengthening, imparting a greater force per muscle fibre (Ebbeling & Clarkson, 1989). The greater mechanical stress from eccentric contractions, in comparison to concentric or isometric-based exercise, changes the structural integrity of the muscle fibers, leading to sarcomere z-line streaming and sarcolemma disruption (Proske & Morgan, 2001). The related damage to the E-C coupling system contributes to muscle soreness and loss of force production capacity and reduced muscle function (White & Wells, 2013). The damage to the sarcolemma makes the fibre more permeable allowing Ca+ overload, which causes further degradation in structural and contractile proteins and further damage to membrane integrity from protease activity (Armstrong, Warren & Warren, 1991; White & Wells, 2013). In addition to fibre structural damage, the associated increase in oedema and phagocytotic activity (cell debris clearance) during the inflammatory response may lead to secondary damage and functional impairment (Merrick, 2002; White & Wells, 2013).

2.4.2. Metabolic stress

It is widely accepted that metabolic stress in active skeletal muscles from prolonged and/or high-intensity exercise, e.g. endurance or interval sports/training, leads to an increased reactive oxygen species (ROS) generation. ROS are thought to play an important role as mediators of EIMD and inflammation (Powers & Jackson, 2008; Sjödin, Hellsten & Apple, 1990; Vollaard, Shearman & Cooper, 2005) and are attributed to a higher oxygen consumption and higher electron leakage within the mitochondria, a potent site for ROS production (Sachdeva & Davies, 2008; Sjödin, Hellsten & Apple, 1990; Vezzoli *et al*., 2014). ROS are highly reactive and can cause chemical chain reactions such as lipid peroxidation, which can damage cell membranes and lead to loss of cell integrity (Sjödin, Hellsten & Apple, 1990). The associated damage to the sarcolemma (Kourie, 1998; Powers & Jackson, 2008; White & Wells, 2013) and structures of the E-C coupling system (Kourie, 1998; White & Wells, 2013). In combination with the accumulation of metabolites from high-intensity exercise, which increases the muscle cells osmolality (McKenzie, *et al*., 1999), oedema and swelling are exacerbated (Yanagisawa *et al*., 2003; White & Wells, 2013). This leads to the abovementioned inflammatory cascade (see section 2.4) leading to secondary damage and functional impairment leading to suboptimal athletic performance (White & Wells, 2013).

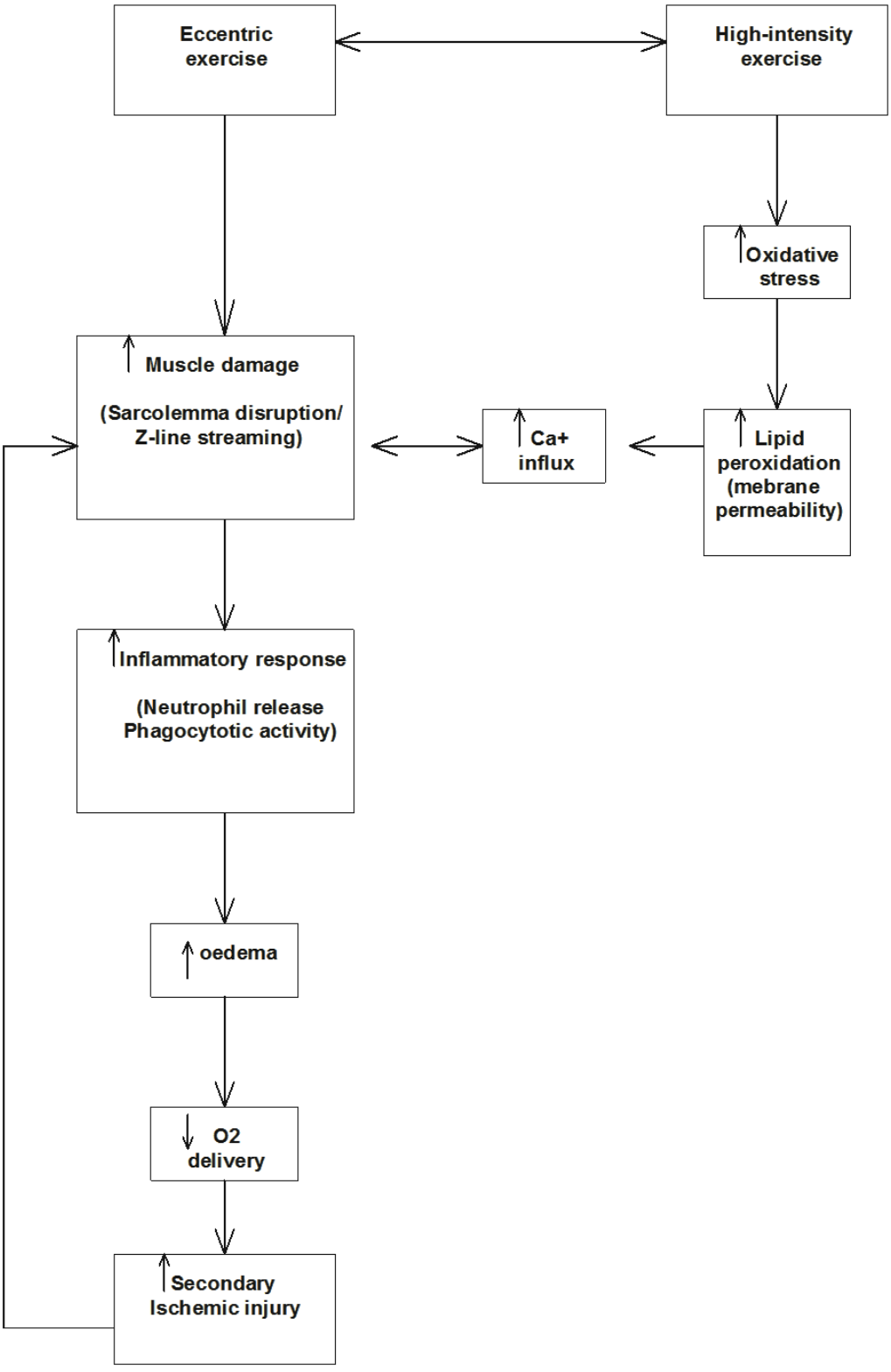

Figure 2.4.1. Simplified illustration of EIMD from mechanical and metabolic pathways.

2.5. RECOVERY MODALITIES

Recently, a lot of attention in the literature has focused on identifying methods to enhance the rate of recovery from exercise, to assist in optimizing subsequent performance. There are several available recovery interventions, which may be selected after exercise, for example, active recovery, stretching, massage, electromyostimulation, compression garments, hyperbaric oxygen therapy, nonsteroidal anti-inflammatory drugs, hydrotherapy, cryotherapy, or a combination of these methods (Barnett, 2006; Cheung, Hume & Maxwell, 2003; Howatson & van Someren, 2008). The majority of these recovery modalities are centered on the recovery from metabolic fatigue and/or EIMD (Barnett, 2006; Cheung, Hume & Maxwell, 2003). Since metabolic fatigue is largely transient (Layzer, 1990), recovery methods that focus on recovery from such stress (e.g. H+, Pi and K+ accumulation) may be seen as being specific to athletic performance that may be repeated on the same day, i.e. competition heats or twice-daily training sessions. It is strategies that predominantly benefit recovery from high-intensity and/or eccentric exercise that causes EIMD, which are applicable for recovery between days and pertinent to many sports.

2.6. COLD WATER IMMERSION AND EXERCISE RECOVERY

Cryotherapy, using ice application, has traditionally been used in the acute treatment and management of soft tissue injury in first aid and post-surgical settings (Bleakley & Hopkins, 2010). The clinical benefit associated with cryotherapy is related to its ability to extract body heat from tissue (Bleakley & Hopkins, 2010). However, despite a lot of recent attention in empirical literature, the underlying physiological mechanisms that underpin its use, still remains to be fully elucidated (Gregson *et al*.,

2011; Halson *et al*., 2008). Cryotherapy is proposed to attenuate the associated inflammatory response, swelling and pain (Smith, 1991) and secondary muscle injury (Merrick *et al*., 1999). Since inflammation is integral in the aeitology of EIMD (Smith, 1991), cooling of exercised limbs has become a popular method to enhance recovery in athletic settings using cold-water immersion (CWI) or whole body cryotherapy (WBC) chambers (Howatson & van Someren, 2008; McGorm *et al*., 2015). The application of post exercise CWI consists of simply immersing the body into water of a reduced temperature to induce beneficial physiological perturbations. CWI may be applied in the field using inflatable baths or bins filled with hosed tap water and ice, or indoors using a specialized facility with a plunge pool or bath maintained at a specific temperature using a cooling unit. The most commonly used CWI temperatures and durations range between 10 to 15 ºC (McGorm *et al*., 2015; Versey, Halson & Dawson, 2013) and 5 to 10 min, respectively (McGorm *et al*., 2015). The immersion depth may vary from localized cooling of a body part (Eston & Peters, 1999) to immersion to the waist (Howatson, Goodall & van Someren, 2009), sternum (Brophy-Williams, Landers & Wallman, 2011), shoulders (Montgomery *et al*., 2008) or neck (Stacey *et al*., 2010).

The majority of CWI research has focused on ameliorating decrements in performance measures, including, force output, time trial time, sprint ability or vertical jump height, after different modes of exercise (Ascensão *et al*., 2011; Bucheit *et al*., 2009, Higgins, Heazlewood & Climstein, 2011); as this may be viewed as the main outcome goal to practitioners in the field. CWI has also been applied after specific muscle damaging protocols, with markers of EIMD and associated DOMS i.e. force output, range of motion measurements, subjective soreness scales,

histological markers and blood levels of myofibre proteins (Eston & Peters, 1999; Howatson, Goodhall & van Someren, 2009; Kuligowski *et al*., 1998) typically measured.

2.6.1. High-intensity exercise and CWI recovery

Over the last decade, a large number of studies have examined the effects of CWI on recovery from high-intensity exercise (Table 2.6.1) with functional and performance measures frequently examined to assess associated physiological and functional deficits. For the purpose of this thesis, high-intensity exercise refers to exercise, which involves a high metabolic cost and some eccentric contractions (i.e. repeated sprints; Leeder *et al*., 2012). CWI applied after short duration maximal intensity exercise, i.e. anaerobic performance (typically repeated within an hour), has consistently been shown to either have a negative effect (Crowe *et al*., 2007; Parouty *et al*., 2010; Schniepp *et al*., 2002) or no observed effect (Buchheit *et al*., 2009; Peiffer *et al*., 2010a) on recovery of peak power, total work or time trial time. However, intermittent exercise protocols, which have a greater endurance component, have shown CWI to have a more positive effect on performance recovery. For example, MVIC (Pournot *et al*., 2011a), total work (Lane & Wenger, 2004), sprint and time trial time (Vaile *et al*., 2008) and repeated intermittent performance (Brophy-Williams, Landers & Wallman, 2011) have all been reported to improve after this type of exercise. In support of CWI being more effective after longer duration protocols, a faster time trial time and increased average power have been reported after a repeated submaximal cycling and time trial protocol (Peiffer *et al*., 2010a). The lower core body temperature observed after the post exercise CWI appeared to help maintain these indices after the subsequent high-intensity

performance (Peiffer *et al*., 2010a). In general, the application of CWI after high intensity exercise has produced varied results on performance outcomes. The effects of CWI remain unclear due to the use of different exercise modalities i.e. anaerobic or endurance exercise, CWI protocols (duration, depth and temperature) and measured dependent variables (Leeder *et al*., 2012). Furthermore, study methodologies have only provided descriptive rather than mechanistic insight in to the effects of CWI on recovery from high-intensity exercise.

Table 2.6.1. The effect of CWI on recovery from high-intensity exercise

Study	Protocol	Subjects	Intervention	Outcome
Bosak *et al*., 2009	5 km running performance trial. Repeated 24 h later	Trained runners	CWI: 12 min in 16 °C. Posture & depth not reported. CON: Passive recovery	↓5 km running time (24 h) ↓Average HR (24 h) ↓Post trial RPE (24 h)
Brophy-Williams *et al*., 2011	Running high-intensity interval session (8 x 3 min at 90 % v- VO_{2max})	Well trained team sport athletes	CWI: 15 min in 15 °C to sternum, posture not reported. (2 CWI trials performed immediately & 3 h post exercise). CON: 15 min seated	<u>Immediate CWI:</u> ↑Yoyo performance (24 h) ↑Perceived recovery (24 h) ↓CRP (24 h) <u>3 h CWI:</u> Likely ↑Yoyo performance Likely ↑perceived recovery Immediate CWI likely ↑Yoyo performance v 3 h CWI
Bucheit *et al*., 2009	1 km cycling time trial in 35°C, 40 % humidity. Repeated 20 min later	Adult cyclists	CWI: 5 min in 14 °C, seated to mid sternum. CON: seated rest in 35 °C & 40 % humidity	↑HR variability indexes before & after exercise bout 2 ↑Perceived recovery
Crowe *et al*., 2007	30 *s* maximal cycling test. Repeated 1 h later.	Strength trained and team sport players	CWI: 15 min in 13-14 °C, seated to umbilical level. CON: 15 min seated rest.	↓Relative peak power ↓Relative total work ↓Exercise La ↓ HR_{peak}
Lane & Wenger, 2004	18 min of high intensity intermittent sprint cycling (12 x 5 *s*, 6 x 10 s and 4 x 15 *s* sprints). Repeated 24 h later.	Physically active	CWI: 15 min in 15 °C leg immersion. Posture not reported. CON: 15 min seated rest.	↑Cycling total work
Parouty *et al*., 2010	100m freestyle swimming time trial. Repeated 30 min later	National level swimmers	CWI: 5 min in 14-15 °C to shoulders in seated position. CON: 5 min seated rest.	↑Swim time ↓HR change & HR peak ↓Perception of recovery ↑Parasympathetic activity vs CON in the 2nd swim.

Peiffer *et al*., 2010a	25 min of cycling at 65 % VO_{2max}, then a 4 km trial in 35 °C (Performed 15 min later)	Well trained cyclists	CWI: 5 min in 14 °C to mid sternum in a seated position. CON: 15 min seated rest in 35 °C.	↑Time trial time ↑Average power ↓Rectal temperature ↓Pre & post exercise bout 2
Peiffer *et al*., 2009a	Cycling time to exhaustion at first ventilatory threshold power in 40 °C.	Trained cyclists	CWI: 5, 10 and 20 min in 14 °C to mid sternum level in a seated position. CON: 20 min seated rest in 24 °C.	No significant difference between measures
Peiffer *et al*., 2009b	90 min of cycling at 80 % of second ventilatory threshold power, then a 16.1 km time trial in 32.2±0.7 °C.	Well trained cyclists	CWI: 20 min in 14 °C to mid sternum in a seated position. CON: 20 min seated rest in 24 °C.	↓MVIC ↓Rectal temperature ↓Skin temperature ↓Decreased femoral vein diameter
Peiffer *et al*., 2010b	1 km time trial in 35±0.3 °C. Repeated 20 min later	Trained cyclists	CWI: 5 min in 14 °C to mid sternum in a seated position. CON: 5 min seated rest in 35 °C.	↓Rectal temperature (all CWI durations) (~1 h) ↓Muscle temperature > 5 min CWI durations) (~1 h)
Pournot *et al*., 2011	Exhaustive intermittent exercise protocol (2 x 10 min separated by 10 min).	Elite football, rugby and volleyball athletes	CWI: 15 min in 10 °C to the iliac crest in a seated position. CON: 15 min seated.	↑MVIC (24 h) ↓Leucocytes (1 h) ↓CK (24 h)
Schniepp *et al*., 2002	0.2-mile cycling sprint. Repeated 15 min later.	Highly trained cyclists	CWI: 15 min in 12 °C to iliac crest, posture not reported. CON: 15 min seated rest.	↓ Peak power ↓ Average power ↓ HR in sprint 2
Stacey *et al*., 2010	3 x 50 kJ all out cycling bouts separated by 20 min.	Habitually active adults	CWI: 10 min in 10 °C to neck level in a seated position. CON: 10 min lying supine.	↑Total leukocytes & neutrophils (1 h) ↑Perceived leg recovery (1 h) ↑Neutrophils during exercise ↓Lymphocytes (1 h post exercise)
Stanley *et al*., 2012	60 min high intensity cycling session (8 x 4 min at 80 % peak power).	Endurance trained cyclists	CWI: 5 min at 14 °C to shoulder level. CON: 10 min seated rest.	↑ Cardiac parasympathetic activity ↓Perceived recovery ↓Leg soreness

Vaile *et al*., 2008	High intensity cycling (66 maximal sprints of 5-15 s, 9 min of time trial efforts). Once per day for 5 days.	Endurance trained cyclists	CWI: 14 min in 15 °C standing upright to shoulder level. CON: 14 min seated rest	↑Sprint performance (days 4 & 5) ↑Time trial performance (across 5 days)
Yamane *et al*., 2006	25 min of cycling at 70 % VO_{2max}, 4 x per week for 4 weeks.	Sedentary students	CWI: 20 min in 5±1 °C. 1 leg immersed (foot out). Performed a 2nd time after 30 min out of water. CON: untreated leg, 20 min rest in room air.	↓VO_{2max} & cycling time during VO_{2max} test
Yamane *et al*., 2006	25 min of cycling at 70 % VO_{2max}, 4 x per week for 6 weeks.	Sedentary students	CWI: 20 min in 5±1 °C. 1 leg immersed (foot out). CON: untreated leg, 20 min rest in room air.	↓VO2max, ventilatory threshold

2.6.2. Eccentric exercise and CWI recovery

In this thesis, eccentric exercise refers to exercise, which involves high mechanical stress, e.g., lengthening/eccentric contractions (Leeder *et al*., 2012). Specific muscle damaging protocols, consisting of eccentric contractions, have been a popular method to examine the effects of CWI on EIMD and DOMS (Table 2.6.2) The majority of these studies have shown CWI to have an inconsistent effect on recovery. For example, resting limb angle, limb circumference, creatine kinase (CK) and perceived muscle soreness have either been shown to decrease (Eston & Peters, 1999, Kuligowski *et al*., 1998; Vaile *et al*., 2008) or not change (Paddon-Jones & Quigley; Sellwood *et al*., 2007) in the days after CWI. Similarly, CWI appears to have little effect on muscle function after these types of protocols, with only one evaluated study reporting MVIC leg strength to be improved (Vaile *et al*., 2008) (Table 2.6.2). Jumping movements, a requisite of several sports, have also been used to study the effects of CWI on recovery from EIMD due to the involvement of a high eccentric

component (stretch-shortening cycle). Again, these studies have shown CWI to have no effect on muscle strength, limb circumference or muscle soreness (Howatson *et al*., 2009; Jakeman et al., 2009). Additionally, CWI has been applied after simulated team sport protocols/matches, which have been used to induce muscle damage via high number of repeated eccentric contractions. In several studies of this type, CWI has improved recovery of muscle function in the days after exercise (Ascensão *et al.,* 2011; Bailey *et al*., 2007; Ingram *et al*., 2009). These findings are in line with associated reported decreases in subjective measures of muscle soreness and blood markers of muscle damage (Ascensão *et al.,* 2011; Bailey *et al*., 2007; Ingram *et al*., 2009; Montgomery 2008; Rowsell *et al*., 2011). Other performance measures, e.g. sprints, vertical jump or time trial times, have shown less consistent results with measures shown to either improve (King & Duffield, 2009; Ingram *et al*., 2009; Montgomery 2008; Rowsell *et al*., 2011; Yeargin *et al*., 2008) not change (Hamlin, 2007; Rowsell *et al*., 2009) or decrease (Higgins *et al*., 2011) after CWI.

The effects of CWI on recovery from eccentric/muscle damaging exercise are confirmed by recent systematic meta-analysis reviews (Leeder *et al*., 2012; Poppendieck *et al*., 2013). These studies confirm that CWI is an effective strategy to alleviate DOMS after muscle damaging exercise (Leeder *et al*., 2012) and can have a positive effect on improving physiological and functional recovery (Poppendieck *et al*., 2013). Whilst the mechanisms underpinning the benefits of CWI on recovery from eccentric based exercise are not fully understood (Leeder *et al*., 2012; Poppendieck *et al*., 2013), inconsistencies between study findings may be related to the differences in water immersion protocols, measures of EIMD and/or the type of preceding exercise.

Table 2.6.2. The effect of CWI on recovery from eccentric exercise

Study	Protocol	Subjects	Intervention	Outcome
Ascensão *et al.,* 2011	Junior friendly football match	Junior national league footballers	CWI: 10 min in 10 °C to iliac crest in a seated position. CON: 10 min in 35 °C (thermoneutral) water to iliac crest in a seated position	↓CK (24, 48 h) ↓CRP (24 h) ↑MVIC (24 h) ↓Muscle soreness (24 h)
Bailey *et al*., 2007	LIST (90 min of intermittent stimulated football activity)	Healthy active adults	CWI: 10 min in 10 °C, seated to the iliac crest. CON: 10 min seated rest.	↑ MVIC (24, 48 h) ↓Mg (1 h) ↓Muscle soreness (24, 48 h)
Eston & Peters, 1999	Concentric and eccentric maximal isokinetic contractions of the elbow flexors (8 sets x 5 reps)	University students	CWI: 15 min in 15±1 °C, exercise arm immersed. CON: No treatment.	↑Relaxed elbow angle (48, 72 h) ↓CK on days 48, 72 h)
Hamlin, 2007	20 m multistage shuttle run followed by repeat sprint test (6 x 5, 10 & 15 m sprint shuttles on 30 *s*)	Development netballers	CWI: 1 min in 8 °C in seated position & 1min standing in air. Performed x 3 on legs. CON: 6 min rest.	No significant difference between measures
Heyman *et al*., 2009	Standardized indoor rock climbing to exhaustion. Repeated 20 min later	Trained climbers	CWI: 5 min in 15±1 °C, 2 min in air seated. Repeated x 3 with arms excluded, hands immersed. CON: seated rest	↑Climbing performance ↓Decreased Tsk ↓Thermal sensation
Higgins *et al*., 2011	1 rugby union game and 2 training sessions per week for 4 weeks	Well trained rugby union players	CWI: 5 min in 10-12 °C to above waistline. CON: passive rest	<u>After 4th game</u> ↓Repeated sprint performance (0 h) ↑Tightness (48 h)
Howatson *et al*., 2009	Drop jumps (5 sets of 20 jumps). Repeated 14-21 days later	Recreationally active	CWI: 12 min in 15±1 °C to the iliac crest. Posture not reported. CON: 12 min seated rest	No significant difference between measures

Ingram *et al*., 2009	Simulated team sport exercise (4 x 20 min of intermittent running), then a 20 m multistage shuttle run test.	Team game experienced	CWI: 5 min in 10 °C, 2.5 min out of water seated, 5 min in 10 °C to umbilicus. Posture in water not reported	↑ MVIC (48 h) ↓muscle soreness (48 h) ↑Repeated sprint ability (48 h)
Jakeman *et al*., 2009	CMJ (10 sets of 10 jumps)	Physically active	CWI: 10 min in 10±1 °C to iliac crest in a seated position. CON: No treatment	No significant difference between measures
King & Duffield, 2009	Simulated netball circuit involving repeat sprints. Repeated 24 h later.	Trained netballers	CWI: 5 min in 9±2 °C to the iliac crest, 2,5 min in air seated. Performed x 2. Posture in water not reported	CWI: ↑Repeated CMJ performance (24 h)
Kuligowski *et al*., 1998	Non-dominant elbow flexor eccentric contractions (5 set x 10 reps at > 1RM concentric)	University students	CWI: 24 min in 13 °C. Exercised arm immersed to mid deltoid level. CON: No treatment.	CWI: ↑Resting elbow flexion ↓Arm soreness
Montgomery *et al*., 2008	Basketball tournament (one 48 min game per day for 3 days)	Development basketball players	CWI: 1 min in 11 °C to midsternal level, 2 min in air. Performed x 5. CON: No treatment	<u>Morning after tournament</u> ↑20 m sprint, ↑Vertical jump ↑Basketball line drill ↑Sit and reach ↓Muscle soreness ↓Fatigue
Paddon-Jones & Quigley, 1997	64 eccentric elbow flexions per arm (8 sets of 8 reps at 110 % of concentric 1 RM)	6 months resistance trained experienced	CWI: 20 min in 5±1 °C, 60 min in air. Arm (exc hand) immersed x 5. CON: No treatment for non-immersed arm.	No significant difference between measures
Rowsell e*t al*., 2011	Football tournament containing 1 match per day for 4 days	Under 17 state level footballers	CWI: 1 min in 10 °C to midsternal level, 1 min out of water seated. Performed x 5. CON: 1 min in 34 °C water to midsternal level, 1 min out of water seated. Performed x 5.	↑Total running time and distance in moderate HR zone ↓Leg soreness & general fatigue

Roswell *et al*., 2009	Football tournament containing 1 match per day for 4 days	Under 17 state level footballers	CWI: 1 min in 10 °C to midsternal level, 1 min out of water seated. Performed x 5. CON: 1 min in 34 °C water to midsternal level, 1 min out of water seated. Performed x 5.	No significant difference between measures
Sellwood *et al*., 2007	Non dominant leg extensions (5 sets x 10 reps at 120 % of concentric 1 RM)	Untrained adults	CWI: 1 min in 5±1 °C to iliac crest in a standing position, 1 min out of water. Performed x 3. CON: 1 min in 24 ±1 °C water (thermoneutral) to iliac crest in a standing position, 1 min out of water. Performed x 3.	No significant difference between measures
Vaile *et al*., 2008	Eccentric leg press protocol (5 sets x 10 reps at 120% of concentric 1RM, 2 sets x 10 reps at 100 % of 1RM)	Strength trained	CWI: 14 min in 15 °C to shoulders in an upright position. CON: 14 min seated rest.	↑ MVIC & loaded ↑ Squat jump peak power ↓Post-exercise thigh circumference ↓CK (24, 72 h)
Yamane *et al*., 2006	Handgrip contraction/relaxation exercise (3 sets x 8RM). 3 x per week for 3 weeks	Sedentary students	CWI: 20 min in 10±1 °C, one forearm immersed (hand out). CON: untreated arm, 20 min rest in air.	↓Handgrip strength & endurance.
Yeargin *et al*., 2006	90 min moderate intensity hilly trial run in 27 °C.	Highly trained heat acclimatized distance runners	CWI: 12 min in 5±1 °C, upper legs to shoulders immersed. CWI: 12 min in 14±0 °C, upper legs to shoulders immersed. CON: 12 min seated rest.	CWI: 14 °C: ↓ Race time CWI 5 & 14 °C: ↓ HR in first half of race

2.7. PHYSIOLOGICAL RESPONSES TO CWI AT REST AND AFTER EXERCISE

The application of CWI after exercise is a popular recovery modality partly due to its ability to rapidly cool whole limbs and large muscle groups. In this sense, CWI has a greater practicality compared with other modes of cryotherapy, such as ice/gel packs and cold air, due to both the large surface area exposed to water and the conductance heat transfer, which is 25 times greater than that of air (Halson *et al*., 2008; Lee *et al*., 1997). There are a number of physiological responses associated with the application of CWI (cryotherapy). However, the two key responses, which are integral to its therapeutic effect, are related to the changes in body temperature (i.e. thermoregulatory) and blood flow (Figure 2.6.1; Yanagisawa *et al*., 2003; Bleakley & Davidson, 2010; Gregson *et al*., 2011; Leeder *et al*., 2012). The beneficial perturbations in these parameters after CWI are proposed to reduce EIMD by ameliorating inflammation and oedema (Thorlacius *et al*., 1998; Lee *et al*., 2005), associated muscle soreness and progression of further secondary injury (Merrick *et al*., 2010; Figure 2.7.1).

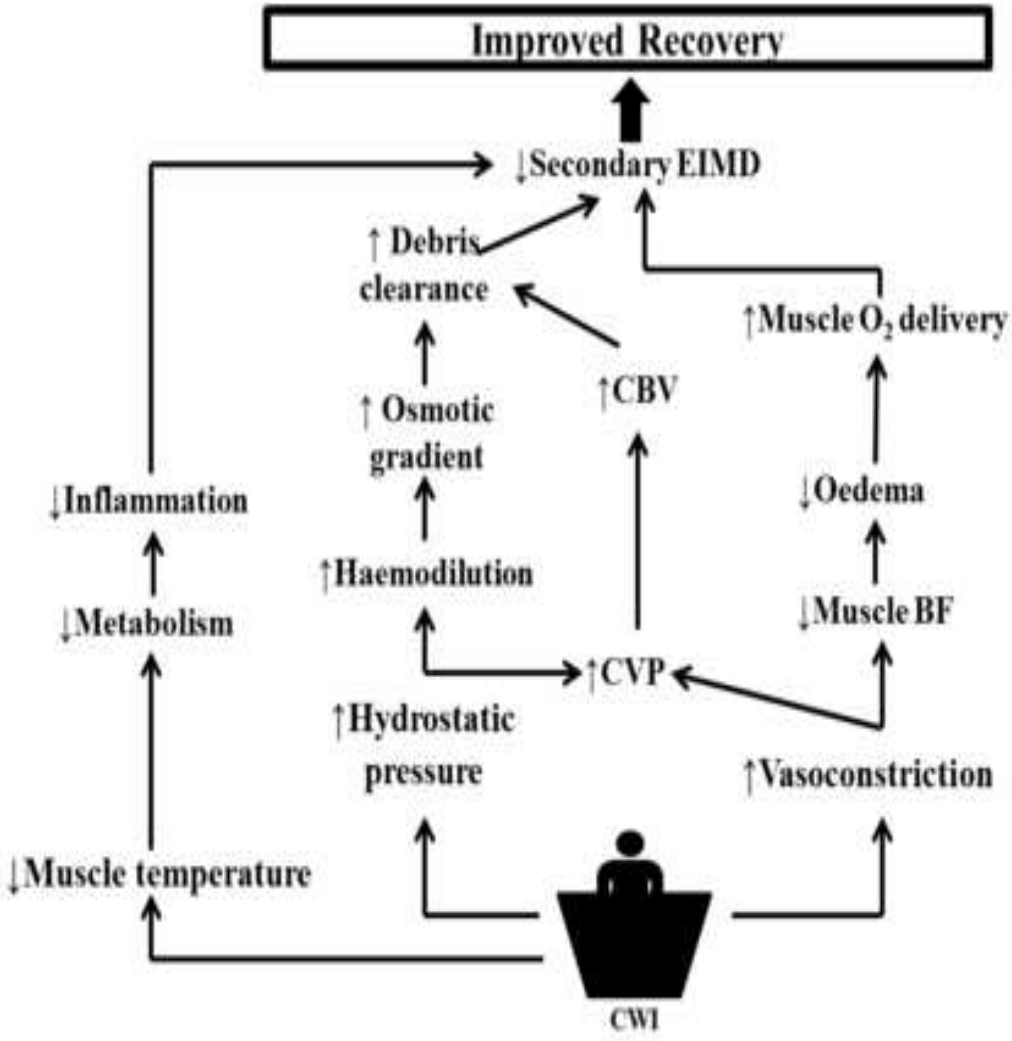

Figure 2.7.1. Possible mechanisms mediating the effects of CWI on EIMD (adapted from Ihsan, Watson & Abiss, 2016).

2.8. THERMOREGULATORY RESPONSES

The application of localized cold or cryotherapy inevitably leads to reductions in tissue temperature, eliciting a number of physiological responses to maintain core body temperature. The following sections discuss the integration of the mechanisms involved and the differences in responses after CWI is applied after rest and exercise.

2.8.1. Thermoregulatory control

The immediate decrease in skin temperature during CWI reduces the skin to environment temperature gradient and limits the rapid heat loss from the body. This is achieved through detection of the cold stimuli via thermosensation to provide a thermoregulatory afferent signal for homeostatic mechanisms to maintain the body at an optimal working temperature (Schepers & Ringkamp, 2009). Temperature information detected by thermoreceptors located in the skin surface feedforward the afferent information to the preoptic anterior hypothalamus (PO/AH), the thermoregulatory center in the brain, to initiate cold defensive thermogenic responses (Morrison, Nakamura & Madden, 2008). Whilst thermoreceptors are also located within the body core structures, in the brain, spinal cord and abdomen, they are not as immediately susceptible to changes in environmental temperature as skin temperature (Morrison, Nakamura & Madden, 2008) and it is therefore the skin, which initially acts as the major sensory input to cold stress.

A decrease in skin temperature facilitates a decrease in skin blood flow to prevent heat loss from the body surface. This is achieved via reflex neural control and is mediated by sympathetic vasoconstrictor nerves releasing norepinephrine, which have an affinity to post-synaptic α1- and α2-receptors on cutaneous arterioles (Charkoudian, 2003; Kellogg, 2006). In addition, local cooling of the skin can decrease skin blood flow via local activation of adrenergic nerves and via greater affinity to post-synaptic α-receptors, independent of an intact sympathetic nerve supply (Charkoudian, 2003; Kellogg, 2006). The maintenance of core temperature is partly achieved through endogenous heat production, which occurs through shivering and non-shivering thermogenesis. Shivering thermogenesis involves rapid, repeated

skeletal muscle contractions to increase metabolism and heat production (Nakamura & Morrison, 2011), whereas non-shivering thermogenesis emanates from metabolism of brown adipose tissue (Cannon & Nedergaard, 2011). These heat generation mechanisms are both mediated by the sympathetic nervous system and the associated release of norepinephrine via stimulation of cold stress (Cannon & Nedergaard, 2011). The thermosensation of cold stress and the appropriate thermoregulatory responses to conserve and generate body heat are consistent with a negative feedback loop to maintain temperature homeostasis (Figure 2.8.1).

2.8.2. Skin temperature

Skin temperature is immediately reduced upon exposure to cryotherapy by heat conduction (Costello *et al*., 2012a; Gregson *et al*., 2011). It is the large temperature heat transfer gradient that exists between the skin and cryotherapy application, i.e. CWI or WBC, which is responsible for the rapid reduction in skin temperature. Skin temperature is reduced during CWI independent of being preceded by rest (Gregson *et al*., 2011; Costello *et al*., 2012a; Costello *et al*., 2014) or exercise (Halson *et al*., 2008; Peiffer *et al*., 2009a; Ihsan *et al*., 2013; Roberts *et al*., 2015a). The magnitude of skin temperature reduction is largely dependent on the water temperature, with greater decreases in skin temperature associated with colder water temperatures (Proulx, Ducharme & Kenny, 2003; Gregson *et al*., 2011). A pattern of gradual increase in skin temperature towards baseline values occurs during the post CWI recovery period (Peiffer *et al*., 2009a; Gregson *et al*., 2011; Costello *et al*., 2012a; Costello *et al*., 2014; Roberts *et al*., 2015a). Under resting conditions, 8 °C CWI applied for a duration of 4-10 min, has been shown to reduce thigh skin temperature from a pre-immersion value of ~30 °C to a minimum of ~15-21 °C during CWI

(Gregson *et al*., 2011; Costello *et al*., 2012a). Similar changes in skin temperature have been observed when CWI is applied after resistance (Roberts *et al*., 2015a) and endurance exercise (Peiffer *et al*., 2009a), respectively. Roberts *et al* (2015a) reported 10 °C CWI applied to the lower body for 10 min after leg isokinetic exercise to decrease thigh skin temperature from ~35 °C to a minimum of ~24 °C at the end of immersion. A comparable magnitude of decrease was observed by Peiffer *et al* (2009a), whom reported that 20 min of 14 °C CWI, applied after 90 min of constant power cycling and a 16.1 km trial in the heat (32 °C), reduced skin temperature from ~32 °C to ~21 °C. These findings suggest that a skin temperature change of approximately ~10-15 °C is achievable when short duration CWI (temperature range of 8-14 °C) is applied after resting and exercise conditions, respectively.

2.8.3. Core body temperature

Core body temperature decreases after exposure to CWI under resting (Gregson *et al*., 2011; Costello *et al*., 2012) and exercise conditions (Peiffer *et al*., 2009a; Peiffer *et al.,* 2009b; Robey *et al*., 2013; Vaile *et al*., 2011). Previous studies have reported that 14 °C CWI, applied up to the sternum for 5-20 min after cycling exercise, significantly decreases (Δ ~1.5-2 °C) pre immersion rectal temperature (T_{rec}) at the end of a post cooling recovery period (Peiffer *et al*., 2009a; Peiffer *et al.,* 2009b; Robey *et al*., 2013; Vaile *et al*., 2011). This is in contrast to 8 °C CWI applied over durations of 4 min (Costello *et al*., 2012) and 10 min (Gregson *et al*., 2011) under resting conditions, which results in a smaller decrease (Δ ~0.2°C-0.3 °C) in T_{rec} during a post cooling recovery period. The disparity in the magnitude of change in T_{rec} between conditions may be partly attributed to the initial and progressive fall in core temperature after exercise *per se* since T_{rec} has been documented to be similar to

CON (rest) trials during and after post-exercise immersion (Peiffer *et al*., 2009a; Peiffer *et al* 2009b). Nonetheless, absolute core temperature values remain higher (above baseline) for a considerable period of time during the post immersion period after exercise i.e. > 20 min (Peiffer *et al*., 2009a; Peiffer *et al* 2009b) compared with CWI applied after rest (Costello *et al*., 2012a; Gregson *et al*., 2011). Interestingly, T_{rec} is reported to fall below baseline values under both resting (Gregson *et al*., 2011; Costello *et al*., 2012a) and exercise (Peiffer *et al*., 2009a; Peiffer *et al* 2009b; Robey *et al*., 2013; Vaile *et al*., 2011) conditions after CWI. This is indicative of the afterdrop phenomenon in core body temperature (Proulx, Ducharme & Kenny, 2003) and is related to the conductive heat transfer from the periphery to the core upon exiting CWI (Webb, 1986; Gagnon *et al*., 2010).

2.8.4. Muscle temperature

Skin cooling leads to decreases in muscle temperature (T_{muscle}) via conductive heat exchange between underlying tissue layers and convective cooling of the limb (Enwemeka *et al*., 2002; Gregson *et al*., 2011). Colder water temperatures cause greater decreases in intramuscular temperature with the magnitude of T_{muscle} change dependent on the thermal gradient, which exists between the muscle and water (Gregson *et al*., 2011; White & Wells, 2013). The change in T_{muscle} is also related to the duration of cold exposure (Meeusen & Lievens 1986), with very large decreases (~18 °C) in T_{muscle} observed after 3 h of CWI (Abramson *et al*., 1966). Under resting conditions, both 10 min of 8 °C (Gregson *et al*, 2011) and 20 min of 10 °C CWI (Myrer, Measom & Fellingham, 1998**)** has been found to decrease superficial (1 cm) T_{muscle} (Δ ~4-5 °C) immediately after immersion. A continued slower rate of reduction (Δ ~1-2 °C) in superficial T_{muscle} was noted during the post CWI recovery periods. In

contrast to superficial muscle depths, Gregson *et al*, (2011) reported deeper (3 cm) T_{muscle} to be similar to baseline values immediately upon exiting CWI, however a significant decrease (Δ ~2 °C) in deep T_{muscle} was observed at the end of a 30 min recovery period. In support of these findings, Costello *et al*, (2012a) reported a similar pattern of change in deep T_{muscle} after 4 min of CWI. Costello *et al's* (2012a) study is significant since it was the first study to compare superficial and deep T_{muscle} against a WBC exposure of equal duration (4 min). The authors reported that there was no difference in T_{muscle} over the 60 min post intervention period. Unfortunately, this study lacked efficacy since CWI is typically applied over a greater duration, i.e. 10-15 min. In addition, the difference in tissue cooling between these two recovery modalities was not established after exercise (i.e. CWI applied after rest).

There are presently a limited number of studies, which have investigated the effects of CWI on post exercise T_{muscle}. After resistance exercise, superficial T_{muscle} (~1-2 cm) has been shown to decrease to the greatest extent (Δ ~6-7 °C) immediately after CWI (Roberts *et al*., 2015a; Roberts *et al*., 2014). In contrast to resting conditions, where a continued post CWI decrease in superficial T_{muscle} occurs (Gregson *et al*., 2011), an increase towards baseline values was documented over a 50 min recovery period. The observation of a slower rate of reduction and/or an increase of T_{muscle} towards baseline values during the post immersion period is consistent with the warmer deeper tissue losing heat to more superficial tissues via conductive and hemodynamic convective heat loss pathways (Enwemeka *et al*., 2002). Unfortunately, deeper (3 cm) T_{muscle} was not reported in the aforementioned resistance exercise studies. However, Peiffer *et al,* (2009b) has observed deep (3 cm) T_{muscle} to follow a similar pattern of decrease to the

aforementioned resting studies when 5 min of 14 °C CWI was applied after a 1 km cycling time trial in the heat.

The effect of CWI on T_{muscle} is important to establish as it also may also influence changes in muscle blood flow (Barcroft & Edholm, 1943) and therefore underpins the amelioration of oedema, secondary injury and functional recovery. Since conductive heat exchange is influenced by the temperature of the water and the body, and is the basis for the abovementioned beneficial perturbations to occur, it is important to establish the impact that different water temperatures can have on these responses. To date, only one study (Gregson *et al*., 2011) has examined the effects that different degrees of CWI can have T_{muscle} responses under resting conditions. In Gregson *et al's* (2011) study, the difference in the magnitude of T_{muscle} responses between the cooling conditions demonstrate the impact that different water temperatures may potentially have on recovery. However, the impact of different degrees of water immersion on these indices after exercise has yet to be fully elucidated.

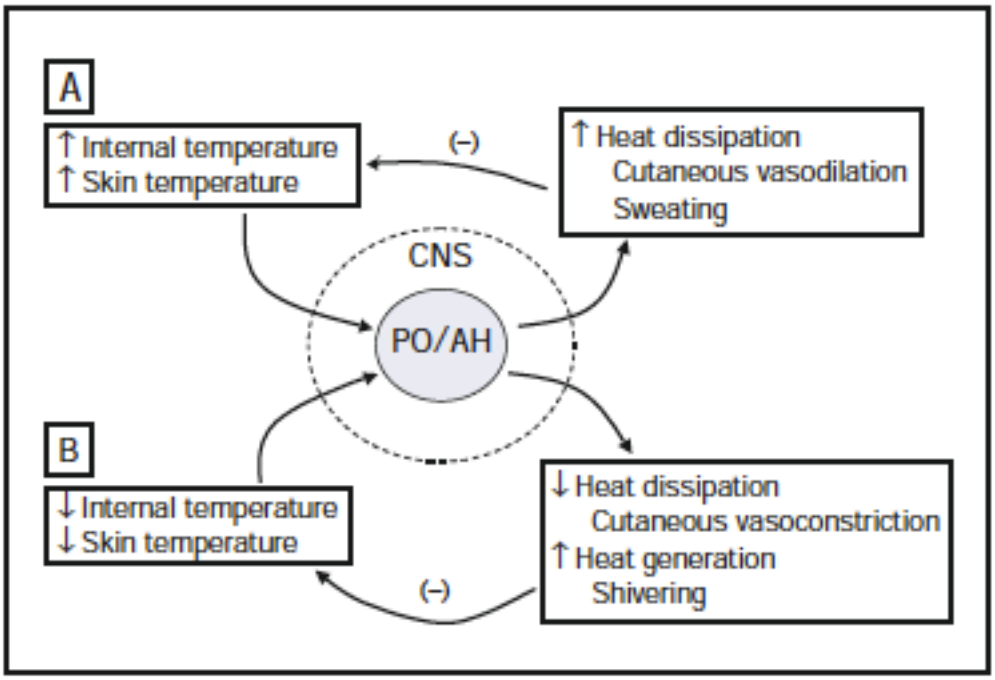

Figure 2.8.1. The negative feedback loop involved in physiologic thermoregulation. The minus signs refer to the correction of the error signal (change in skin and or/ internal temperature) by the appropriate effector response (adapted from Charkoudian, 2003).

2.9. CARDIOVASCULAR RESPONSES

The cardiovascular system is under the influence of both neuronal and humoral components of the autonomic nervous system (ANS) that control automated body functions including heart rate (HR), blood pressure (BP) and metabolism (Carnethon & Craft, 2008). The ANS has sympathetic and a parasympathetic components, which act antagonistically to control body functions. It is the sympathetic arm of the ANS that is primarily responsible for responding to physical and psychological stimuli (Carnethon & Craft, 2008). The principal function of the ANS is to maintain blood supply to the major body organs of the body; this is achieved through the control of BP via modulation of cardiac output ($\dot{Q}$) and systemic resistance (i.e. blood pressure = cardiac output x total peripheral resistance or BP = $\dot{Q}$ x TPR). The application of CWI

to the body imparts a challenge to the ANS to respond to the independent stimuli of cold stress and hydrostatic pressure. This may represent an autonomic conflict during water immersion at a decreased temperature. For example hydrostatic pressure at thermoneutral temperatures increases $\dot{Q}$ and decreases TPR, whereas colder water temperatures have been reported to decrease $\dot{Q}$ but increase TPR (Wilcock, Cronin & Hing, 2006). The following sections discuss the cardiovascular responses to CWI with reference to the positive pressure of water where relevant.

2.9.1. Heart rate

Thermoneutral water immersion generally leads to a decreased HR via hydrostatic related central hemodynamic changes (Wilcock, Cronin & Hing, 2006). However, the HR response is largely dependent on water temperature. Indeed, whilst Šrámek *et al*, (2000) reported that both 20 °C and 32 °C head out water immersion decreased HR by ~15 %, a colder 14 °C immersion caused a ~5 % increase in HR. In contrast, Bonde-Peterson, Schultz-Pederson & Dragsted, (1992) observed 15 °C CWI applied to a similar depth to decrease HR by 15 % compared with a control (CON) (non-immersion) trial. The inconsistency in the magnitude of HR response to water immersion *per se* has been related to competition between physiological feedback systems (Wilcock, Cronin & Hing, 2006) and is also likely also to provide an explanation for the disparity in documented HR responses after CWI. The sympathetic increase in mean arterial pressure (MAP), related to CWI, activates arterial baroreceptors, which brings about a reflex slowing of the heart to prevent high BP levels. In opposition, the increase in central blood volume (CBV), which is accentuated with immersion above hip level, stimulates atrial stretch receptors and activates a neural reflex called the Bainbridge reflex that increases HR (Wilcock,

Cronin & Hing, 2006). Despite the varied HR response during CWI, it is clear that colder water temperatures initially increase HR due to the well-established cold shock response in the first few seconds of immersion (Tipton, 1989). Therefore, the reported increases in HR during CWI are likely to be associated with an increased sympathetic activity and a decreased vagal outflow (Mourot, Bouhaddi & Regnard, 2009).

Numerous studies have demonstrated that HR, which is elevated due to exercise prior to immersion, is decreased following CWI exposure (Schniepp *et al*., 2002; Bailey *et al*., 2007; Pointon *et al*., 2012; de Oliveira-Ottone *et al*., 2014; Roberts *et al*., 2015a). For example, Bailey *et al*, (2007) reported that 10 min of 10 °C CWI applied to the iliac crest after a 90 min shuttle run decreased HR from 107 bpm to 94 bpm, however CWI had no effect on HR compared with the CON group (non-immersion). Similarly, a consistent decrease in HR has been observed during a passive recovery period when CWI has been applied between two bouts of exercise (Pointon *et al*., 2012; Vaile *et al*, 2008). The decrease in exercise HR in the recovery period after CWI may be attributed to the competition between sympathetic and parasympathetic nervous activity, with CWI thought to increase the latter (during the post CWI period), leading to a reduction in HR (Buccheit *et al*., 2009; Stanley *et al*., 2012; Stanley *et al*., 2013).

2.9.2 Stroke volume and cardiac output

The temperature and hydrostatic effect of water influences $\dot{Q}$ via related changes in HR and stroke volume (SV). Under resting conditions, Park, Choi and Park, (1999) observed immersion to the neck at temperatures of 30 °C and 34.5 °C, respectively, to increase $\dot{Q}$ by ~50 % compared with a non-immersion trial. The authors attributed the increase in $\dot{Q}$ to an increased cardiac preload and SV from the water hydrostatic

pressure; indeed central venous pressure (CVP) increases with skin cooling (Cui *et al*., 2005; Wilson *et al*., 2007). In support of these findings, Lin, (1984) whom used weighted averages to compare 8 studies, which all included immersion to the neck versus non-immersion trials, found an approximate 29 % and 24 % increase in SV and $\dot{Q}$, respectively. In contrast, Bonde-Peterson, Schultz-Pederson & Dragsted, (1992) investigated the effects of CWI at rest (30-40 min CWI at 15 °C to the sternum) and reported that despite a 19 % increase in SV, there was concomitantly relatively little change in $\dot{Q}$ due to a decreased HR response. In agreement, Roberts *et al* (2015a) reported that after isokinetic resistance exercise, 10 min of 10 °C CWI to the level of the umbilicus, led to a decrease in $\dot{Q}$ with a concomitant decrease in SV. These abovementioned findings are difficult to compare since greater immersion depths are associated with a higher SV and $\dot{Q}$ during thermoneutral immersion (Wilcock, Cronin & Hing, 2006). Consequently, the comparison of differences in SV and $\dot{Q}$ responses between resting and exercise conditions is difficult to interpret due to the varied selection of water immersion depths in previous studies and the pre-immersion cardiovascular status. However, the failure to observe significant increases in $\dot{Q}$ after CWI may be attributed to the redirection of blood flow from the periphery to the core, which enhances cardiac efficiency, reduces cardiovascular strain (Stanley *et al.,* 2014; Vaile *et al*., 2011), and in some instances is associated with a lower HR when CWI is applied after exercise (Stanley *et al.,* 2014). The increase in CVP and CBV facilitates haemodilution after CWI (Ihsan, Watson & Abiss, 2016; Johansen *et al*., 1995; Johansen *et al*., 1997; Stocks *et al*., 2004). This may assist the transport of the abnormal increase in interstitial fluid from muscle fiber trauma, i.e. oedema, and the removal of cellular debris into the central circulation (Ihsan, Watson & Abiss, 2015; Wilcock, Cronin & Hing, 2006), enhance metabolite clearance including P^+ and H^+

(Yanagisawa *et al*., 2003) and limit secondary injury (Merrick *et al*., 1999) (Figure 2.7.1).

2.9.3 Arterial blood pressure

The assessment of arterial BP is typically undertaken by immersing the hands in cold water, i.e. the cold pressor test. (Victor, Liembach & Seals, 1987). Utilizing this method, Kregel, Seals & Callister, (1992) documented the BP response to 3 min of one-handed immersion at various levels of temperature (28, 21, 14, 7 and 0 °C) and observed greater increases in both systolic and diastolic pressure compared with CON values during immersion in the colder water temperatures (0-7 °C). A similar BP response has been reported during 30-40 min of 15 °C CWI to the sternum under resting conditions, with increases in systolic, diastolic and MAP observed compared with a CON trial (Bonde-Peterson, Schultz-Pederson & Dragsted, 1992). The measurement of arterial BP responses has seldom been included in CWI exercise research studies. However, an increase in systolic BP has recently been reported after 10 min of 10 °C CWI has been applied after isokinetic resistance exercise (Roberts *et al*, 2015a).

2.9.4. Limb blood flow

Several previous studies which have assessed the limb blood flow response to CWI under resting and exercise conditions, have used the venous occlusion plethysmography (VOP) technique and reported either no change (Fiscus, Kaminski & Powers, 2005) or a decrease (Barcroft & Edholm, 1943; Vaile *et al*., 2011) in measured limb blood flow. Similar findings have been observed when VOP has been used to measure limb blood flow after local ice pack/gel pack application (Baker &

Bell, 1999; Karunakara, Lephart & Pincivero, 1999; Taber *et al*., 1992). The VOP method entails keeping arterial inflow to the limb intact whilst venous outflow is temporarily arrested to allow strain gauge measures of changes in limb circumference and calculation of limb blood flow (Rådegran, 1999). Whilst reliable estimates of changes in whole blood flow can be calculated using VOP, this technique only provides discontinuous blood flow measurements i.e. 1 measure every 5-10 *s* (Woodman *et al*., 2001) and can not differentiate between skin and muscle blood flow. Since cooling induces changes in cutaneous blood flow (CBF) (Gregson *et al*., 2011; Sendowski *et al*., 1997), it is likely that VOP measures don't accurately reflect changes in underlying muscle blood flow after CWI (Gregson *et al*., 2011).

The limb blood flow response to lower body CWI has recently been assessed using the Doppler ultrasound technique to insonate the superficial femoral artery alongside simultaneous measures of CBF by laser Doppler flowmetry (Gregson *et al*., 2011). The Doppler ultrasound method uses measures of blood velocity and arterial diameter to determine arterial blood flow. This technique has advantages over VOP since it permits continuous (30 Hz) absolute blood flow measurements. The combined assessment of femoral arterial blood flow (FABF) and skin blood flow provides an indirect estimate of muscle perfusion. Using this method, Gregson *et al*, (2011) compared cold (8 °C) and cool (22 °C) water immersion temperatures under resting conditions, and found that despite FABF decreasing to a similar extent, CBF was observed to be higher in the colder water. This finding inferred muscle perfusion was less in colder water and highlighted the impact CBF can have on the limb blood flow response. However, to date no study has used this technique to investigate the limb blood flow response to different water temperatures after exercise.

2.9.5. Muscle blood flow

In addition to the recovery benefits arising from temperature related reductions in tissue temperature, CWI is proposed to decrease microvascular blood flow within an injured muscle (Lee *et al*., 2005 Thorlacius *et al*., 1998), which may reduce oedema (Dolan *et al*., 1997) and secondary injury (Merrick, 1999) (Figure 2.7.1). Unfortunately, the measurement of muscle blood flow is somewhat problematic since there is currently no clearly defined technique to measure muscle blood flow in humans (Casey, Curry & Joyner, 2008). The gold standard approach to measuring changes in muscle blood flow is via positron-emission tomography (PET) technique. This method uses intravenous [^{15}O] H_2O radiowater to measure blood flow in tissues where there is an exchange of water molecules i.e. where exchange of nutrients and oxygen occur. The PET technique can distinguish between muscle and skin blood flow and can provide insight into capillary level blood flow (Heinonen *et al*., 2011). Because PET scanners are few in number and are very expensive to operate, regional (systemic) and local techniques have been used to provide indirect estimates of muscle blood flow (Casey, Curry & Joyner, 2008; Rådegran, 1999). Unfortunately, many of the available methods have inherent limitations or are not suitable to be used during repeated measures CWI studies.

Bonde-Peterson, Shultz-Person & Dragsted, (1992) found no change in forearm blood flow after 30-40 min of 15 °C CWI to the neck using the local isotope clearance technique. Despite this method allowing the measurement of muscle perfusion by observing the clearance rate of an injective (i.e. Xe^{133}), it has been shown to underestimate muscle blood flow (Rådegran, 1999). Additionally, the collection of repeated measurements of blood flow during CWI are difficult to obtain due to the

injection artifact delaying repeated measures by approximately 10 min (Rådegran, 1999). More recently, near infrared spectroscopy (NIRS) has been used to estimate changes in local muscle blood flow after CWI, (Ihsan *et al*., 2013; Roberts *et al*., 2015a), WBC (Selfe *et al*., 2014) and ice pack application (Yanagisawa *et al*., 2007). This method utilizes the different absorption of near infrared light to determine concentration changes in oxygenated haemoglobin, deoxygenated haemoglobin and total haemoglobin to estimate local muscle blood flow (Casey, Curry & Joyner, 2008; Rådegran, 1999). Utilizing the NIRS method, muscle blood volume has been shown to decrease after CWI has been applied after both endurance (Ihsan *et al*., 2013; Stanley *et al*., 2014) and resistance (Roberts *et al*., 2015a) modes of exercise. However, the NIRS technique only provides indirect estimates of changes in blood volume within the microcirculation and not blood flow *per se*. Furthermore, the contribution of myoglobin to the NIRS signal cannot be differentiated from haemoglobin and the signal may be confounded when marked changes in skin blood flow arise such as with heating and cooling (Davis *et al*., 2006).

The NIRS method has also recently been used to investigate changes in muscle blood volume after exposure to WBC (the day after exercise; Selfe *et al*., 2014). To date, this is the only study that has attempted to examine the changes in muscle perfusion after WBC; unfortunately comparisons in the limb blood flow response between CWI and WBC methods were not addressed. This is important to establish since it will assist practitioners in selecting an optimal recovery strategy based on the greatest magnitude of blood flow reduction between the two modes of whole limb cryotherapy.

Since decreases in microvascular blood flow and associated recovery benefits are related to reductions in tissue temperature, and water temperature is inherently linked to its reduction (Barcroft & Edholm, 1943), it is important to establish the impact that different water temperatures may have on these responses. Gregson *et al* (2011) study demonstrates the impact that different water temperatures may potentially have on recovery. Nevertheless, the impact of different degrees of water immersion on these indices after exercise has yet to be elucidated. The limb blood flow response to different modes of cryotherapy after rest and exercise, are presented in Table 2.9.1.

Table. 2.9.1. Effects of various cryotherapy methods on whole limb blood flow

Study	Measurement Site	Exercise Type	Cooling Method	Duration	Method of Blood Flow Measurement	Δ Blood flow
Baker & Bell, 1999	Calf	Rest	Ice-pack	20-min	VOP	**No change**
Barcroft & Edholm, 1943	Forearm	Rest	CWI	120-min	VOP	**Decrease**
Bonde-Peterson, Shultz-Pederson & Dragsted, 1992	Forearm	Rest	CWI	30-40-min	Xe^{133}	**No change**
Fiscus, Kaminski & Powers, 2005	Calf	Rest	CWI	20-min	VOP	**No change**
Gregson *et al*., 2011	Thigh	Rest	CWI	2 x 5-min (separated by 2 min)	Doppler ultrasound and laser Doppler flowmetry	**Decrease**
Ihsan *et al*., 2013	Thigh	Continuous & intermittent running	CWI	15-min	NIRS	**Decrease**
Karunakara, Lephart & Pincivero, 1999	Forearm	Rest	Ice-pack	20-min	VOP	**Decrease**
Roberts *et al*., 2015a	Thigh	Isokinetic exercise	CWI	10-min	NIRS	**Decrease**
Selfe *et al*., 2014	Thigh	Rest	WBC	1-3-min	NIRS	**Decrease**
Selkow *et al*., 2011	Calf	Rest	Ice-pack	10-60-min	Contrast enhanced ultrasound	**No change**
Stanley *et al*., 2014	Thigh	Cycling exercise	CWI	5-min	NIRS	**Decrease**
Taber *et al*., 1992	Ankle	Rest	Cold gel-pack	20-min	VOP	**Decrease**
Thorsson *et al*., 1985	Thigh	Rest & running	Ice-pack	20-min	Xe^{133}	**Decrease**
Vaile *et al*., 2011	Thigh & forearm	Cycling exercise	CWI	15-min	VOP	**Decrease**
Yanagisawa *et al*., 2004	Calf	Ankle dorsiflexion exercise	Ice-pack	~5-min	MRI	**Decrease**
Yanagisawa *et al*., 2007	Calf	Rest	Cooling-pad	30-min	NIRS	**Decrease**

2.10. NEGATIVE EFFECTS OF CRYOTHERAPY

2.10.1. Freezing and non-freezing cold injury

Whilst the physiological responses to cryotherapy may benefit post-exercise recovery, there are also negative effects associated with its use, especially when the body is exposed to extreme temperatures over prolonged periods. A side effect of reducing tissue temperature is frostbite. Skin sensation is lost at 10 °C, with further cooling increasing the viscosity of vascular contents, microvascular constriction, trans-endothelial plasma leakage and closure of arteriovenous shunts. This may lead to extracellular ice crystal formation, cellular damage and eventually frostbite (Imray, Grieve & Dhillon, 2009). A less well-documented non-freezing cold injury may also occur from exposure to wet and cold conditions just above freezing temperatures (0-15 °C) (Imray *et al*., 2011). This type of injury may lead to localized sensory neuropathy upon tissue rewarming and the onset of pain, which may be prolonged (months) (Imray, Grieve & Dhillon, 2009). Although poorly understood, it is thought that this type of injury is related to direct injury to the microvascular endothelium that supply blood to nerve, fat and muscle cells (Imray *et al*., 2011).

2.10.2. Adaptation versus recovery

In humans, it has recently been shown that regular CWI application after resistance exercise may attenuate long-term gains (12 weeks) in muscle mass and strength by blunting the activation of key proteins and satellite cells in skeletal muscle (Roberts *et al*., 2015b). It has also recently been demonstrated that CWI application after a bout of resistance exercise, does not decrease neutrophil or macrophage counts compared with active recovery (Peake *et al*., 2016). This finding suggests that CWI may not be effective in reducing the inflammatory response after muscle damaging exercise.

However, more work is required to confirm this conclusion in humans. On the contrary, CWI has been shown to have a positive impact on the muscle adaptive response when applied after endurance exercise via over-expression of transcriptional peroxisome proliferator-activated receptor coactivator-1 alpha (PGC-1a), which is thought to enhance mitochondrial biogenesis (Ihsan *et al*., 2014; Ihsan *et al*., 2015; Joo *et al*., 2015). The benefits of cryotherapy may therefore be dependent upon the timing and purpose of its use, i.e., adaptation versus recovery. For example, reducing oedema and swelling and increasing movement may be beneficial for rapid recovery before an exercise bout or competitive fixture in the short-term (e.g. within days). Alternatively, if the main objective of frequent cryotherapy is for future competition (e.g. weeks away), caution may be used dependent on the nature of the exercise.

2.11. SUMMARY

CWI is a widely used post exercise recovery method to assist athletes in maintaining optimal performance, especially during periods of high volume training or competition when muscle fatigue and/or EIMD may be encountered. The effects of CWI on exercise performance are inconsistent, with CWI likely to be effective in the recovery from high-intensity exercise, which involves a high eccentric component and leads to muscle damage and DOMS. The associated reductions in tissue temperature and muscle blood flow are thought to be integral to ameliorating inflammation, oedema and secondary injury from high intensity and muscle damaging exercise. Previous attempts to study the effects of CWI on muscle blood flow have been limited due to the selection of VOP and NIRS methods. However, recently, simultaneous measurements of changes in FABF and CBF have enabled assessment of indirect estimates of muscle blood in response to different degrees of water immersion under resting conditions (Gregson *et al*., 2011). Nevertheless, the limb blood flow response

to different degrees of water immersion temperatures after endurance and resistance exercise currently remains unknown. Furthermore, it remains to be elucidated whether colder water temperatures elicit greater physiological (thermoregulatory and limb blood flow) benefits to post-exercise recovery. Additionally, it is unknown whether post exercise CWI (water cooling) is a more effective recovery strategy compared with WBC (air cooling), based on changes to key physiological responses.

CHAPTER 3

GENERAL METHODOLOGY

3.1. INTRODUCTION

The present chapter describes the measurement techniques used within this thesis for the collection of thermoregulatory and limb blood flow measures and provides a description of the water immersion protocol. The participant cohort and general methodologies are outlined and the reliability of selected methods are briefly discussed.

3.1.1. Location of testing

All measurements were taken in a climate-controlled laboratory, with the ambient temperature controlled at 22 – 24 °C to avoid the effect of ambient temperature on physiological responses (Cracowski *et al*., 2006) and at the same time of day in order to avoid the circadian variation in internal body temperature (Reilly & Brooks, 1990). Participants remained semi-reclined on a bed during all pre and post exercise measurements.

3.1.2. Participants

Healthy recreationally active male participants aged between 18-38 yrs were recruited for all studies. This sample population is relevant (i.e. age and activity) to athletes who may use post-exercise CWI as a recovery aid to enhance subsequent performance. The participants were familiarized with all experimental procedures and associated risks and gave their written informed consent to participate (Appendix 1 & 2). The research ethical committee of Liverpool John Moores University approved all experimental studies and related procedures.

3.1.3. Anthropometry

Participant's stature was measured whilst standing in a Frankfurt plane using a stadiometer (Seca, Birmingham, U.K.). Nude body mass was recorded using precision calibrated scales (Seca, Birmingham, U.K.). All measurements were undertaken on their first visit to the laboratory.

3.1.4. Dietary and physical activity controls

Prior to all experimental testing, participants recorded nutritional and fluid intake (Appendix 3). This record was photocopied and returned to permit them to repeat their preparation for the remaining trials. They also consumed 5 $mL \cdot kg^{-1}$ of water 2 h before arriving at the laboratory. Participants were asked to refrain from exercise and alcohol for at least 24 h, caffeine for at least 8 h and were not allowed food within 3 h of the commencement of testing.

3.2. EXPERIMENTAL DESIGN

Specific muscle damaging exercise protocols can lead to inflammation and confound post exercise limb blood flow measurements between conditions, due to the protective effect of performing a single bout of muscle damaging exercise (Howatson & van Someren, 2008). Therefore, cycling exercise, which has limited eccentric involvement, was selected to assess the impact of cooling on the limb blood flow response after endurance exercise (Chapter 5 & 7). Similarly, in order to limit muscle damage after resistance (squat) exercise (Chapter 6), participants were selected upon their exercise history, i.e. regular squat exercise. In addition, participants completed the resistance protocol one week prior to experimental testing to reduce the magnitude of muscle damage and the associated inflammatory response, which might influence

limb blood flow. This provides a protective repeated bout effect (Howatson & van Someren, 2008) and reduces the potential for any order effect. Since it was not the intention to induce an inflammatory response, performance measures were not taken to measure the impact of cooling on EIMD. In this thesis, the cycling exercise protocol was used to attain a core temperature of 38 °C prior undertaking the cooling interventions. At this core temperature, the rate of rise in skin blood flow is markedly attenuated despite further significant increases in core temperature (Kellogg *et al*., 1993). This temperature allows for a larger heat exchange between the skin and the cooling intervention compared with lower internal temperatures.

3.3. THERMOREGULATORY MEASUREMENTS

3.3.1. Core body temperature measurements

Core body temperature was measured by recording T_{rec}. Participants self-inserted a rectal probe (A/S Krondalve 9 DK-2610, Ellab, Rodovre, Denmark) 15 cm beyond the anal sphincter, connected to an electronic measuring system (CTF 9004, ELLAB). T_{rec} was measured continuously, with the probe remaining in place inside the rectum until the end of experimental testing. The probe was unattached from the electronic measuring system when participants were required to exit the laboratory. All rectal data were recorded every 2 *s* and averaged over a 2 min period at each measurement point.

3.3.2. Skin temperature measurements

Skin temperature was measured at four sites on the body using skin thermistors (Ellab, Rodovre, Denmark), which were connected to an electronic measuring system (CTF 9004, Ellab, Rodovre, Denmark). The Tsk data were recorded every 2 s and averaged over a 2 min period at each measurement time point. The sites of skin temperature measurement were:

1) Chest, below the sternal notch (Tsk_{chest})
2) Left Forearm, anterior surface, midway between the elbow and wrist ($Tsk_{forearm}$)
3) Left thigh, anterior surface, midway between the hip and knee (Tsk_{thigh})
4) Left calf, lateral surface, midway between the knee and ankle (Tsk_{calf})

In addition to local skin temperature measurements, mean skin temperature (Tsk_{mean}) was calculated using the equation of Ramanathan (1964):

$$Tsk_{mean} = 0.3(Tsk_{chest} + Tsk_{forearm}) + 0.2(Tsk_{thigh} + Tsk_{calf})$$

3.3.3 Muscle temperature measurement

T_{muscle} was assessed using a needle thermistor inserted into the vastus lateralis (13050, Ellab, Rodovre, Denmark). Thigh skinfold thickness was measured using Harpenden skinfold calipers (HSK BI, Baty International, West Sussex, United Kingdom) and divided by 2 to determine the thickness of the thigh subcutaneous fat layer over each participant's vastus lateralis (Enwemeka *et al*., 2002). The needle thermistor was then placed at a depth of 3 cm plus one-half the skinfold measurement for determination of

deep T_{muscle} (3 cm). The thermistor was then withdrawn at 1 cm increments for determination of T_{muscle} at 2 cm and 1 cm below the subcutaneous layer.

3.4. CUTANEOUS BLOOD FLOW MEASUREMENT

The laser Doppler probes were calibrated using a calibration device (PF 1000, Perimed Instruments, Jarfalla, Sweden), which included a motility standard solution. The range of the laser Doppler device is 0-999 perfusion units. The probes were clamped to produce no change in received wavelength and placed in the motility standard solution and preset to 0 PU. The solution uses Brownian motion, the random motion of particles suspended in a fluid, to provide a standardized perfusion value (e.g. Doppler shift) equivalent to a perfusion of 250 PU ± 5 % at an ambient temperature of 22 °C (Perimed, 2015). This procedure was repeated prior to each experimental trial. RBC flux was used as an index of skin blood flow via laser Doppler flowmetry (Periflux System 5001, Perimed Instruments, Jarfalla, Sweden). The skin measurement sites were pre shaved and prepared by cleaning with an alcohol swab and allowed to dry. The laser Doppler probes (PROBE 455, Perimed, Suffolk, United Kingdom) were attached to the skin using double sided ring adhesive and secured in place with Micropore tape, to the mid-anterior thigh, midline, halfway between the inguinal line and the patella, and on the calf, left of the midline, in the region of the largest circumference. Once affixed, the probes were not removed. Participants lay supine for at least 20 min prior to baseline measurements being recorded to allow equilibrium of blood volume throughout the body and for instrumentation. The laser Doppler flowmetry data were converted from perfusion units to cutaneous vascular conductance (CVC) units (AU) by the ratio of laser Doppler flux to MAP (CVC = laser Doppler flux/MAP x 100) and expressed as a

percentage change from pre immersion i.e. pre cooling values. When expressed as a percentage change from baseline to maximum, CBF has an intra-subject coefficient variation (CV) of 4 %, providing an acceptable confidence in the repeatability of this technique (Hodges, 2005, unpublished Ph.D.).

3.5. LIMB BLOOD FLOW MEASUREMENT

3.5.1. Femoral artery blood flow measurement

A 15 MHz multi-frequency linear array transducer attached to a high-resolution ultrasound machine (Acuson P50, Siemens, Germany) was used to measure femoral artery diameter (D) and mean blood velocity (MBV). The images were taken at the superficial femoral artery in the proximal third of the left leg approximately 3 cm distal to the bifurcation (Figure 3.5.1). This position was marked on the skin for ultrasound head repositioning during the remaining measures for accuracy and consistency. Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. Continuous and synchronized pulsed wave Doppler velocities were also obtained using the ultrasound machine. Data were collected using an insonation angle of 60° and each measurement was recorded for 2 min. Analysis of diameter was performed using custom designed edge-detection and wall-tracking software as described previously (Thijssen *et al*., 2011; Woodman *et al*., 2001) which provides simultaneous and continuous measurements of arterial diameter and blood flow velocity. The assessment of blood flow velocity uses the edge detection algorithm to assess the peak velocity envelope from the Doppler gate. From these data the software calculates mean blood flow (MBF; the product of cross-sectional area and blood flow velocity) at 30 Hz. The edge-detection and wall-tracking software is semi-automated and provides diameter measurements, which are

considerably more repeatable (coefficient of variation = 6.7 %) than manual methods and are associated with less observer error (Green *et al*., 2002; Woodman *et al*., 2001). This method of blood flow assessment is closely correlated with actual flow through a phantom arterial flow system (Woodman *et al*., 2001). All data were written to file and retrieved for analysis in the custom designed analysis package. Resting diameter, blood velocity and blood flow were calculated as the mean of the data collected over a 20 s period of each 2 min scan for statistical analysis. Using the MBF and MAP data, femoral vascular conductance (FVC) was calculated as the ratio of blood flow/MAP.

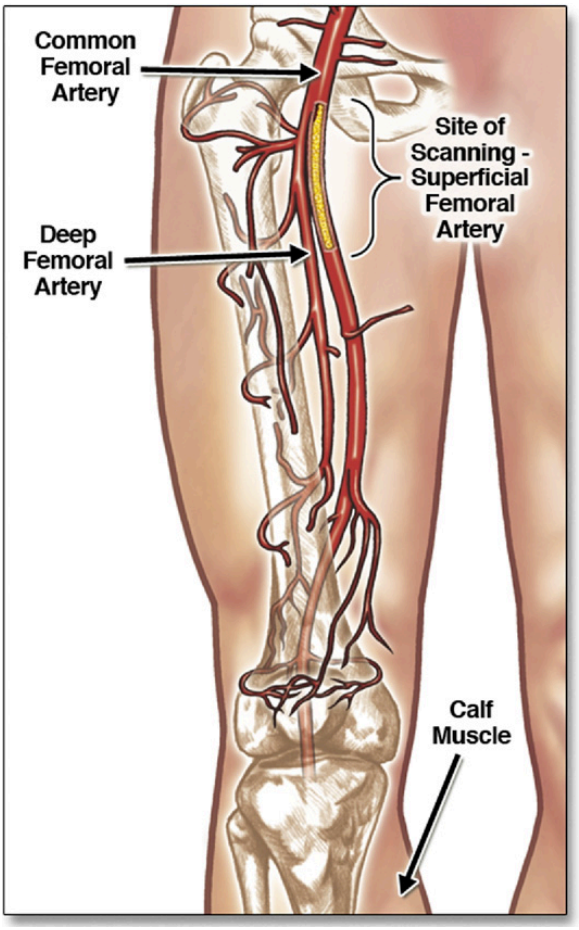

Figure 3.5.1. Location of the transducer placement on the superficial femoral artery (adapted from McDermott *et al*., 2011).

3.6. CARDIO-VASCULAR MEASUREMENTS

3.6.1. Heart rate measurement

HR was continuously measured every 5 *s* using a telemetry chest belt and HR monitor (S610; Polar Electro Oy, Kempele, Finland). Technical specifications state that this method has an accuracy of ±1 % or ±1 bpm under steady state conditions.

3.6.2. Mean arterial pressure measurement

Arterial BP was measured noninvasively via automated brachial auscultation (Dinamap, GE Pro 300V2, Tampa, Florida, USA), and MAP was calculated as [Diastolic + (0.333 x (Systolic-Diastolic))]. Technical specifications state that this method of BP measurement has an accuracy of ≤ 5±8 mmHg.

3.7. PSYCHO-PHYSIOLOGICAL MEASUREMENTS

3.7.1. Thermal comfort

Subjective thermal comfort was assessed using a 9-point thermal ratings scale based on descriptors of thermal sensations (Young *et al*., 1987) ranging from ‘unbearably cold’ to ‘unbearably hot’ (Table 3.7.1).

Table 3.7.1. Ratings of thermal comfort

Rating	Description
0	Unbearably Cold
1	Very Cold
2	Cold
3	Cool
4	Comfortable
5	Warm
6	Hot
7	Very Hot
8	Unbearably Hot

3.7.2. Shivering

Subjective shivering was assessed using a 4-point scale (Wakabayashi *et al*., 2006), ranging from 'no shivering' to 'heavy shivering' (Table 3.7.2).

Table 3.7.2. Ratings of subjective shivering

Rating	Description
1	No Shivering
2	Slight Shivering
3	Moderate Shivering
4	Heavy Shivering

3.7.3. Ratings of perceived exertion

Raring of perceived exertion (RPE) was taken following exercise using a 15-point Borg scale, (Borg, 1970). The category ratio scale is shown below (Table 3.7.3)

Table 3.7.3. Borg scale for participant's ratings of perceived exertion

Rating	Description
6	No Exertion At All
7	Extremely Light
8	
9	Very Light
10	
11	Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

3.8. COLD-WATER IMMERSION

Participants were raised from a bed in a semi-recline position using an electronic hoist (Bianca, Arjo Ltd, Gloucester, United Kingdom) and lowered into the water tank (ECB, Gloucester, U.K.) to the iliac crest for 10 min. At the end of immersion, participants were returned to the bed using the electronic hoist and remained in a semi reclined position. The use of the hoist to raise and lower the participants was important to avoid the effect of muscle activation on FABF and other physiological measures (Figure 3.8.1).

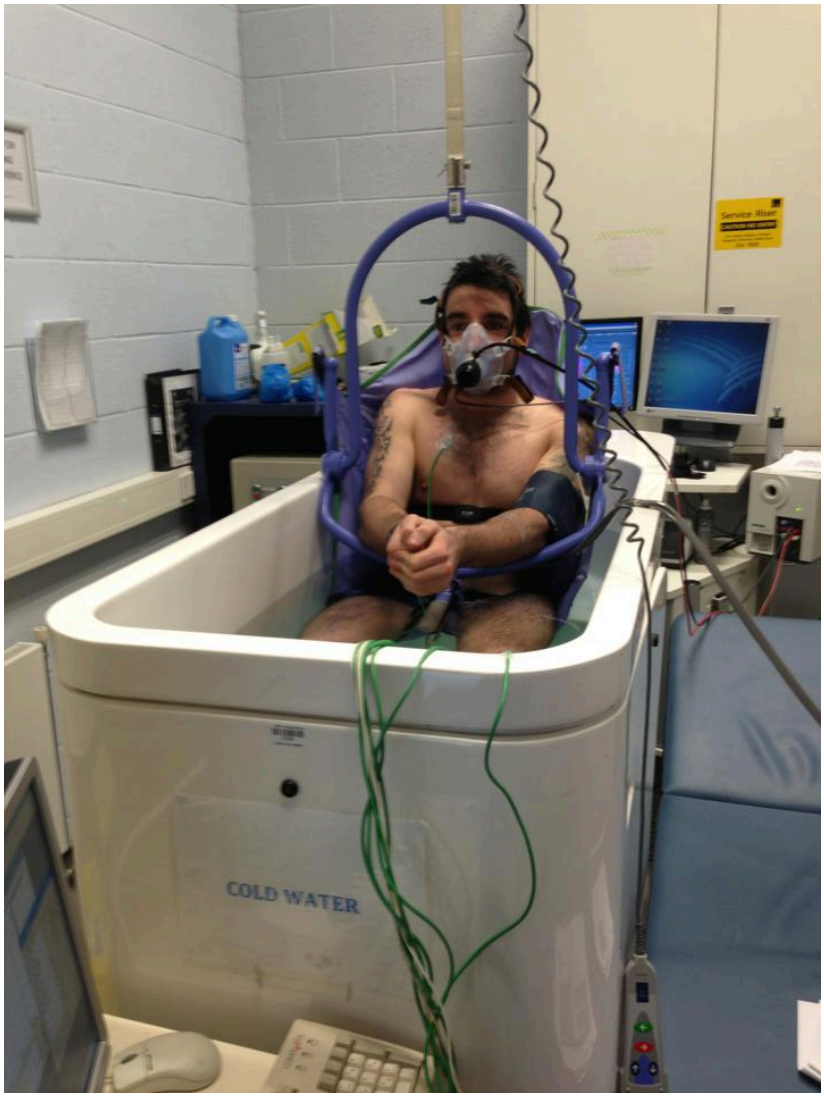

Figure 3.8.1. Illustration of water immersion.

CHAPTER 4

RELIABILITY OF DUPLEX DOPPLER ULTRASOUND IN THE ASSESSMENT OF SUPERFICIAL FEMORAL ARTERY BLOOD FLOW

4.1. INTRODUCTION

Despite the widespread use of CWI as a post-exercise recovery strategy, the underlying physiological mechanisms, which may benefit the recovery process, remain unclear. It has been postulated that cooling an injured muscle leads to a reduction in the delivery of microvascular blood flow, resulting in attenuated edema, inflammatory events and DOMS (Lee *et al*., 2005; Thorlacius *et al*, 1998). A reduction in deep muscle blood flow therefore appears central to any beneficial physiological and functional perturbations associated with CWI. This has led to several investigative studies (Gregson *et al*., 2011; Ihsan et al., 2013; Vaile *et al*., 2011), using various techniques, to attain limb blood flow measures after CWI.

Previous attempts to evaluate the influence of CWI on whole limb blood flow have relied upon VOP (Fiscus *et al.,* 2005; Vaile *et al*., 2011) or NIRS (Ihsan *et al*., 2013; Roberts *et al*., 2015a) techniques. VOP can be reliably used to measure relative changes in whole limb blood flow at rest (Casey *et al.,* 2008), however, this method does not distinguish between muscle and skin blood flows (Gregson *et al*., 2011; Wissler, 2008). Alternatively, NIRS only allows indirect estimates of relative changes in blood volume within the muscle microcirculation, and not blood flow *per se.* In addition, the NIRS signal is confounded when changes in skin blood flow arise e.g. heating, cooling, exercise (Davis *et al*, 2006). In comparison, insonation of a main conduit artery using duplex Doppler ultrasound permits estimates in changes in limb blood flow via collection of noninvasive quantitative measures of blood velocity and vessel cross-sectional area (see section 3.4.1). In combination with simultaneous measures of skin blood flow, this method has been used to provide indirect estimates of changes in muscle blood flow (Gregson *et al.,* 2011).

The assessment of measurement error is of principal concern when establishing the changes in an observed value between repeated measures, i.e. retest reliability (Hopkins, 2000). The reliability of a test refers to an acceptable level of agreement between repeated tests within a practically relevant timeframe (Atkinson and Nevill, 1998). Factors that influence reliability include any systematic or random changes in the mental or physical state of the individual between trials. The protocol and measurement device used to collect the data may also contribute to the variability of the measurements. A test with poor reliability will be unsuitable for tracking changes in the fatigue status of the athlete (Hopkins, 2000).

Whilst there is an absence of studies, which have assessed the within-day reliability of duplex Doppler ultrasound on vascular indices, investigations of between-day measurements have yielded highly reproducible between-day CVs. Under resting conditions, brachial artery D CVs have ranged between 0.4 % - 6.5 % (Shoemaker, Pozeg & Hughson, 1996; Berry, Skyrme-Jones & Meredith, 2000; Woodman *et al.,* 2001; De Roos *et al.,* 2002; De Roos et al., 2003; de Goot *et al.,* 2004; Meirelles *et al.,* 2007), with comparable CVs of 3 % (Dinenno *et al.,* 1999) and 1.5 % (de Goot *et al.,* 2004) reported in the femoral and superficial femoral arteries, respectively. In contrast, greater CVs have been found for between-day measurements of MBV (9 % - 13.2 %) (Shoemaker, Pozeg & Hughson, 1996; Dinenno *et al.,* 1999) and MBF (10 % - 24 %) (Dinenno *et al.,* 1999; Green *et al.,* 2002; de Goot *et al.,* 2004). These observations provide important insight into the origin of the sources of measurement error when undertaking vascular blood flow assessments.

4.2. PURPOSE

The aim of this study was to determine the within and between-day reliability of FABF using duplex Doppler ultrasound. This will provide a basis for estimating samples sizes in future work which will focus on the influence of CWI on whole limb blood flow. It was hypothesized that duplex Doppler ultrasound would provide reliable within and between-day measures of FABF.

4.3. MATERIALS AND METHODS

4.3.1. Participants

Eight recreationally active male volunteers (mean ± s: age, 275.6 years; height, 1.77±0.1 m; weight, 75.1±11.2 kg) were studied.

4.3.2. Experimental protocol

Prior to arrival at the laboratory, all participants were asked to adhere to the pretest dietary and exercise controls (see section 3.1.4). Upon arrival at the laboratory, participants remained rested in a semi-reclined position for a 20-min period to stablise physiological status (Olive, McCully & Dudley 2002), wearing shorts and a t-shirt. Subsequently, two duplex Doppler ultrasound scans of the superficial femoral artery (see section 3.4.1), separated by a 5 min period, were recorded to measure within-day superficial femoral artery D, MBV and MBF. Participants then attended the laboratory on a second visit (within a week of the first test day) for a single between-day scan under the same experimental conditions. MAP (see section 3.5.2) was recorded at the end of each 2 min scan to allow calculation of FVC (see section 3.4.1). A schematic illustration of the experimental design is shown in Figure 4.3.1.

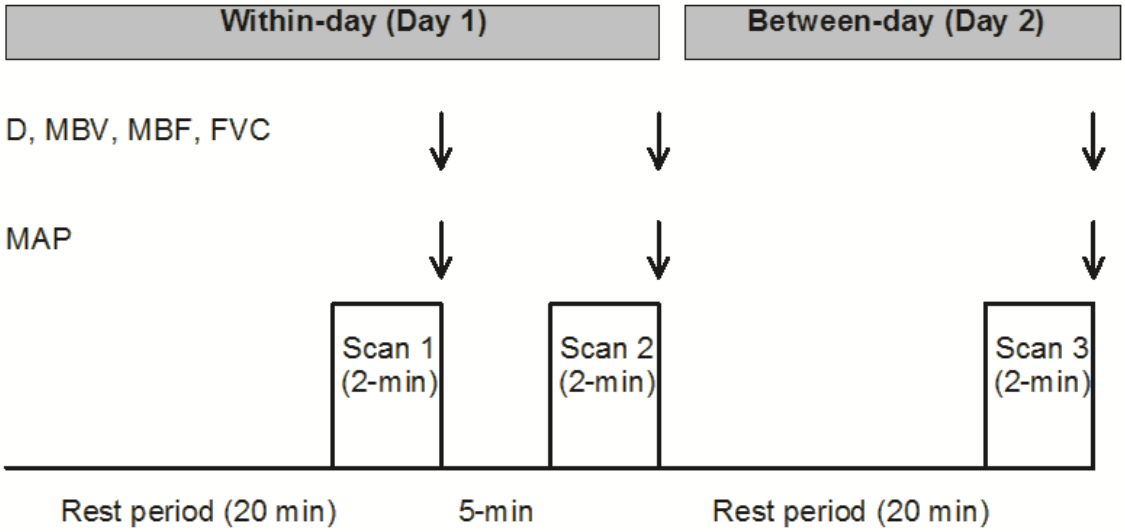

Figure 4.3.1. A schematic of the experimental design.

4.3.3. Statistical analysis

The mean (SD) systematic bias [and associated 95 % confidence interval (CI)] between scan 1 and 2 (within day) and scan 1 and 3 (between day) for the measurements of MAP, D, MBV, MBF and FVC was first quantified using a paired t-test. Random error between repeated tests was quantified with the within-subjects SD [standard error of measurement (SEM)] and CV. The % CV for each variable was determined by dividing the SEM with the pooled mean. Correlations, which collapse different components of bias, as well as random error between and within assessors, have been criticized in the literature for obfuscating separate sources of variability (Atkinson & Nevill, 1997; Nevill & Atkinson, 1998). Statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, IL). The alpha level for the evaluation of statistical significance was set at $P < 0.05$. All data are displayed as mean ± SD.

4.4 RESULTS

4.4.1. Superficial arterial Diameter

There was no difference in D measurements during within-day scans (Scan 1, 0.66±0.08 cm; Scan 2, 0.65±0.08 cm; $P = 0.59$) or between-days scans (Scan 1, 0.66 ± 0.08 cm; Scan 3, 0.68±0.08 cm; $P = 0.23$). The calculated SEM for D was 0.03 cm (CI, -0.05 to 0.07 cm) and 0.1 cm (CI, -0.09 to 0.04 cm) for within and between-days, respectively. When expressed as percentage CV, a 4 % value was observed for within-day scans with a similar 5 % variability calculated for scans between-days (Table 4.4.1).

4.4.2. Mean Blood Velocity

MBV measurements were not different during within-day scans (Scan 1, 263.57±70.86 $cm \cdot min^{-1}$; Scan 2, 231.95±56.60 $cm \cdot min^{-1}$; $P = 0.06$) or scans between-days (Scan 1, 263.57±70.86 $cm \cdot min^{-1}$; Scan 3, 270.14±104.42; $P = 0.71$). The calculated SEM for MBV was 28.30 $cm \cdot min^{-1}$ (CI, -25.05 to 88.3 $cm \cdot min^{-1}$) and 34.70 $cm \cdot min^{-1}$ (CI, -75.86 to 62.73 $cm \cdot min^{-1}$) for within and between-days respectively. The percentage CV for MBV was 11 % for within-day measurements, with a similar variability (13 %) found between-days (Table. 4.4.1).

Table 4.4.1. Arterial measures within and between-day scans (n = 8)**.**

	D (cm)	MBV ($cm \cdot min^{-1}$)	MBF ($mL \cdot min^{-1}$)	FVC (AU)	MAP (mmHg)
Scan 1	0.66	263.57	88.19	1.01	87
(SD)	(0.08)	(70.86)	(19.34)	(0.23)	(3)
Scan 2	0.65	231.95	76.39	0.86	88
(SD)	(0.08)	(56.60)	(17.33)	(0.17)	(5)
Scan 3	0.68	270.14	92.81	1.05	89
(SD)	(0.08)	(104.42)	(25.50)	(0.33)	(7)
WD SEM	0.03	28.30	13.30	0.15	2
(95% CI)	(-0.05 to 0.07)	(-25.05 to 88.30)	(-14.79 to 38.40)	(-0.15 to 0.45)	(-6 to 4)
BD SEM	0.1	34.70	17.75	0.21	3
(95% CI)	(-0.09 to 0.04)	(-75.86 to 62.73)	(-40.12 to 30.88)	(-0.45 to 0.37)	(-9 to 5)
WD %CV	4.37	11.44	16.16	15.87	2.76
(95% CI)	(2.64 to 8.14)	(7.27 to 22.39)	(10.58 to 32.56)	(10.58 to 32.56)	(1.98 to 6.11)
BD %CV	5.03	12.98	19.61	20.05	4.12
(95% CI)	(3.31 to 10.18)	(8.60 to 26.46)	(13.22 to 40.71)	(13.22 to 40.71)	(2.64 to 8.14)

Mean ± SD values are presented. WD, within day; BD, between day; mean blood velocity; MBF, mean blood flow; FVC, femoral vascular conductance; MAP, mean arterial pressure.

4.4.3.Mean Blood Flow

MBF was similar during within-day scans (Scan 1, 88.19±19.34 $mL \cdot min^{-1}$; Scan 2, 76.39±17.33 $mL \cdot min^{-1}$; P = 0.12) and between-days scans (Scan 1; 88.19±19.34 $mL \cdot min^{-1}$; Scan 3, 92.81±25.50 $mL \cdot min^{-1}$; P = 0.62). The calculated SEM was 13.30 $mL \cdot min^{-1}$ (CI, -14.79 to 38.40 $mL \cdot min^{-1}$) and 17.75 $mL \cdot min^{-1}$ (CI, -40.12 to 30.88 $mL \cdot min^{-1}$) for within and between-days respectively. The percentage CV for within-day MBF measurements was 16 % with a CV of 20 % observed between days (Table. 4.4.1).

4.4.4.Superficial femoral Artery conductance

FVC was similar during within-day scans (Scan 1, 1.01±0.23 AU; Scan 2, 0.86±0.17 AU; P = 0.09) and between-days scans (Scan 1; 1.01±0.23 AU; Scan 3, 1.05±0.33 AU; P = 0.70). The calculated SEM was 0.15 AU (CI, -0.15 to 0.45 AU) and 0.21 AU (CI, -0.45 to 0.37 AU) for within and between-days respectively. The percentage CV for within-day FVC measurements was 16 % with a CV of 20 % observed between days (Table. 4.4.1).

4.4.5. Mean arterial Pressure

MAP was similar during within day scans (Scan 1, 87±3 mmHg vs. Scan 2, 88±5 mmHg; P = 0.49) and between-days scans (Scan 1, 87±3 mmHg vs. Scan 3, 89±7 mmHg; P = 0.30). The calculated SEM for within and between-days MAP was 2 mmHg (CI, -6 to 4 mmHg) and 3 mmHg (CI, -9 to 5 mmHg), respectively. When expressed as percentage CV, within-day values of 3 % and between day values of 4 % were observed (Table. 4.4.1).

4.5. DISCUSSION

The main findings of the present study demonstrate that the measurement of MBF and FVC indices, using the duplex Doppler ultrasound method, provides an acceptable degree of measurement error both within and between-days scans. These findings suggest that the duplex Doppler ultrasound is a reliable method to permit future investigations into the effects of CWI on limb blood flow.

In the present study, MBF values were similar both within and between-days. The measurement of MBF yielded relatively small within (13.30 $mL \cdot min^{-1}$) and between-

day (17.75 mL·min^{-1}) SEM values indicating acceptable reliability for measures of MBF. This observation is further supported by the relatively narrow 95 % CI indicating acceptable measurement variability (random error) and the small mean differences in MBF, demonstrating negligible bias both within and between-day scans. The CV in MBF (~16-20 %) compares favorably with previous between-day observations (~10-18 %) reported in the femoral artery (Dinenno *et al.,* 1999; de Goot *et al.,* 2004) and the brachial artery (Green *et al*., 2002) using the Doppler ultrasound method. Because FVC is the product of MBF/MAP, the small variability observed in MAP for this parameter, led to similar levels of measurement error and repeatability compared with MBF. Whilst there is currently an absence of studies, which have investigated the within-day reliability of duplex Doppler measurements of MBF, the present findings are comparable to within-day assessments of forearm and calf VOP blood flow measurements (CV, ~11-13 %), respectively (Roberts, Tsao & Breckenridge, 1986). In contrast, repeated forearm VOP blood flow measures collected periodically over several weeks (i.e. between-day) have been shown to result in slightly higher values (CV ~25 %; Cooke & Dzau, 1997) than presently observed (~16-20 %).

The present data indicated that D and MBV measures were similar both within and between-days. In agreement with previous brachial artery studies (Hijmering *et al.,* 2001; De Roos *et al.,* 2003), the within-day scans showed very little variability in D measures (~4 %). A small variability was also noted between-days (~5 %) and is similar to previous observations (~0.4-6.5 %) (Shoemaker, Pozeg & Hughson, 1996; Berry, Skyrme-Jones & Meredith, 2000; Woodman *et al.,* 2001; De Roos *et al.,* 2002; De Roos *et al*., 2003; de Goot *et al.,* 2004; Meirelles *et al.,* 2007). In the current

study, MBV measurement error was ~11 % for within-day measurements, which was closely matched when scans were performed between-days (~12 %). This variability in MBV was within the range of ~9-13 % of previously reported between-day resting measurements (Shoemaker, Pozeg & Hughson, 1996; Dinenno *et al.,* 1999) and suggests little measurement error between the scans.

The relatively small variation in MBF may partially be explained by the inherent sources of error related to the duplex Doppler ultrasound assessment of blood flow. Because MBF is calculated from the vessel cross sectional area, any error in the measurement of arterial diameter produces an error in MBF (Gill, 1985). Although diameter edge detection wall tracking was used in the present study, Shoemaker, Pozeg & Hughson (1996) highlighted how a small 0.1 mm error in the placement of diameter measurement calipers could result in ~2.5 – 3 % CV when the diameter is within the range of 4 mm. Additionally, operator error in obtaining a B mode image, with clear vessel wall features may affect the accurate measurement of vessel diameter. Nonetheless, the relatively small measurement error for this parameter indicated adequate technical use of the transducer and diameter imaging. The ability to produce a good image of a peripheral vessel wall requires the vessel to be at right angles to the ultrasound beam (Gill, 1985; Thrush & Hartshone, 2005). However, no Doppler signal is obtained when the angle of insonation is at right angles to the direction of blood flow. Therefore, a compromise exists when obtaining an optimal angle of the transducer beam for the imaging of a vessel to measure artery diameter and obtaining a Doppler trace for MVC (Gill, 1985; Thrush & Hartshone, 2005). Consequently, there is a reduced sensitivity to detect returning signals, which can result in large errors occurring in the measurement of MBV. In an attempt to reduce

the MBV measurement error and keep any error constant between measurements, an insonation angle of 60° was selected during this study (Nelson & Pretorius, 1988; Thrush & Hartshone, 2005). In the present study, it is likely that the greater measurement error observed for the estimates of MBF, compared with other vascular parameters, reflected the substantial beat-to-beat variability of blood velocity (Eriksen *et al.,* 1990).

4.5. SUMMARY

The results of this study demonstrate that the duplex Doppler ultrasound method has an acceptable absolute reliability to measure MBF and FVC indices under resting conditions post-exercise. The observation of relatively small SEM and CV indicate a small variability both within and between-days scan measurements, respectively. These findings permit future limb blood flow investigations after CWI using duplex Doppler ultrasound measurements under resting conditions (i.e. data not collected during exercise) and provide a basis for estimating participant sample size in future studies.

CHAPTER 5

INFLUENCE OF COLD-WATER IMMERSION ON LIMB AND CUTANEOUS BLOOD FLOW AFTER ENDURANCE EXERCISE

Communication: Poster presentation at the Annual American College of Sports Medicine conference, San Francisco, June 2011.

Publication: Mawhinney et al. (2013) Influence of cold-water immersion on limb and cutaneous blood flow after exercise. Med Sci Sport Exer, 45(12), 2277-2285. (Appendix 4).

5.1. INTRODUCTION

Localized cold or cryotherapy is frequently applied after acute soft tissue injury in an attempt to minimize the inflammatory response, local edema, swelling and pain, and therefore enhance recovery from soft tissue injury (Bailey *et al*., 2007). Since inflammation is also integral in the development of EIMD (Smith, 1991), whole limb cooling via CWI is now increasingly applied after exercise in order to alleviate some of the physiologic and functional deficits associated with exercise-induced muscle damage (Bailey *et al*., 2007; Vaile *et al*., 2008).

The physiological effects of cryotherapy are thought to be partly underpinned by reductions in microvascular blood flow to the injured muscle (Lee *et al*., 2005; Thorlacius *et al*., 1998), which subsequently reduces edema and the induction of inflammatory events (Bleakley, McDonough & MacAuley, 2004; Dolan *et al*., 1997). Whole limb CWI is therefore likely to be effective by virtue of its effect on deep muscle blood flow (Gregson *et al*., 2011; Lee *et al*., 2005). Recently, simultaneous measurement of skin and limb blood flow has been assessed using laser Doppler flowmetry and high-resolution duplex ultrasound, respectively, to provide an indirect estimate of muscle blood flow in the lower limbs in response to cold (8 ^{o}C) and cool (22 ^{o}C) water immersion at rest (Gregson *et al*., 2011). Immersion in both cold and cool water promoted similar reductions in whole limb blood flow but increased blood flow to the skin in the cold (8 ^{o}C) water. This suggests that colder water temperatures may induce greater reductions in muscle blood flow at rest and may therefore be more effective in the treatment of EIMD and injury rehabilitation.

It is well documented that the vascular response to sympathetic stimulation is blunted during exercise and whole body heat stress compared with rest (Flouris *et al*., 2008; Tschakovsky *et al*., 2002; Wilson, Cui & Crandall, 2002). Reduced vasoconstrictor responsiveness in the skin when cold (8 °C) water immersion is applied after exercise compared with rest may therefore lead to similar changes in CBF to those associated with cool (22 °C) water. Such information has important implications for treatments guidelines because the application of CWI frequently occurs immediately following exercise, when core body and local limb temperatures are elevated. To date, only a few studies have investigated the blood flow response to post exercise CWI (Ihsan *et al*., 2013; Roberts *et al*., 2015a; Vaile *et al*., 2011). However, no attempt has been made to compare the effects of different degrees of cooling on these responses. Previous observations are either limited by their use of VOP (Vaile *et al*., 2011), which is highly sensitive to motion artifact and is limited to the assessment of whole limb blood flow (Rådegran, 1999). Alternatively, observations (Ihsan *et al*., 2013; Roberts *et al*., 2015a) are limited by the use of NIRS, which provides indirect estimates of relative changes in blood volume within the microcirculation. In addition, the NIRS signal is confounded under conditions in which marked changes in skin blood flow arise (e.g., exercise, heating and cooling; Davis *et al*., 2006).

5.2. PURPOSE

The purpose of the present study was to examine the effects of cold (8 °C) and cool (22 °C) water immersion on CBF and FABF responses after exercise using laser Doppler flowmetry and high-resolution duplex ultrasound. It was hypothesized that that whole-limb immersion in 8 °C and 22 °C water would decrease FABF to a similar extent after exercise, but colder water would reduce T_{muscle} to a greater extent.

5.3. MATERIALS AND METHODS

5.3.1. Participants

Twelve recreationally active men were studied (mean ± SD: age, 25.5±4.7 yrs; height, 1.8±0.1 m; mass, 78.4±7.7 kg, BSA, 2.0±0.1 m^2; $\dot{V}O_{2peak,}$ 47±8 mL · kg^{-1} · min^{-1}). Participants completed all the pretest criteria prior to testing (see section 3.1.2, 3.1.3 and 3.1.4).

5.3.2. Preliminary testing

Before any experimental trials, each participant completed a maximal incremental cycling protocol on a cycle ergometer (Daum ergo bike, Premium 8i, Germany) while simultaneous breath-by-breath ($\dot{V}O_2$) measurements were recorded (Oxycon Pro, Jaeger, Germany). The cycling protocol commenced at 75 W and was increased 25 W every 2-min until volitional exhaustion was reached. Peak power output was derived as the highest power output attained at this point. Maximal oxygen uptake ($\dot{V}O_{2peak}$) (mL · kg^{-1} · min^{-1}) was recorded as the highest 30 *s* average recorded before volitional exhaustion.

5.3.3. Experimental protocol

Each participant was required to complete a submaximal cycle ergometer protocol, followed by a 10 min period of immersion in 8 °C and 22 °C water and seated rest (CON). The water temperature and immersion protocol were based on data frequently reported in the literature (Gregson *et al*., 2011; Wilcock, Cronin & Hing, 2006). The different temperature trials were conducted in a counterbalanced order, 1 week apart. On arrival at the laboratory, the participant's self-inserted a rectal probe and a HR monitor was positioned across the chest. Participants were then laid in a supine

position for 30 min on a bed (next to the water tank) for the attachment of instrumentation and to stabilize physiological status (Olive, McCully & Dudley, 2002), wearing training shorts and a tracksuit top. After baseline measurements, participants cycled at 70 % $\dot{V}O_{2peak}$ until a core temperature of 38 °C was attained. Participants then returned to a supine position for 10 min to enable pre-immersion measurements to be taken. Participants were subsequently immersed into the water tank for a duration of 10 min (see section 3.7) or remained suspended above the bed (CON). At the end of immersion, participants were returned to the bed using the electronic hoist and remained in a supine position for a period of 30 min in the laboratory under an ambient temperature of 22 °C-24 °C. The attainment of 38 °C core temperature was selected since this leads to a rise and plateau in skin blood flow to maximize heat exchange with the water.

T_{rec} (see section 3.2.1), Tsk_{chest}, $Tsk_{forearm}$, $Tsk_{thigh,}$ and Tsk_{calf} and Tsk_{mean} (see section 3.2.2), HR (see section 3.5.1) and thigh and calf CBF (see section 3.3) were continuously monitored at baseline, before immersion, and throughout the 30 min post-immersion period. T_{muscle} (see section 3.2.3) was recorded at baseline, immediately before and after immersion and 30 min after immersion. FABF (see section 3.4.1) and MAP (see section 3.5.2) were measured at baseline, before immersion, immediately after immersion, and at 10 min intervals throughout the 30 min post-immersion period. Perceived thermal comfort (see section 3.6.1) and shivering (see section 3.6.2) were also recorded at the same time points. All pre and post-immersion measurements were made in a supine position. A schematic illustration of the experimental design is shown in Figure 5.3.1.

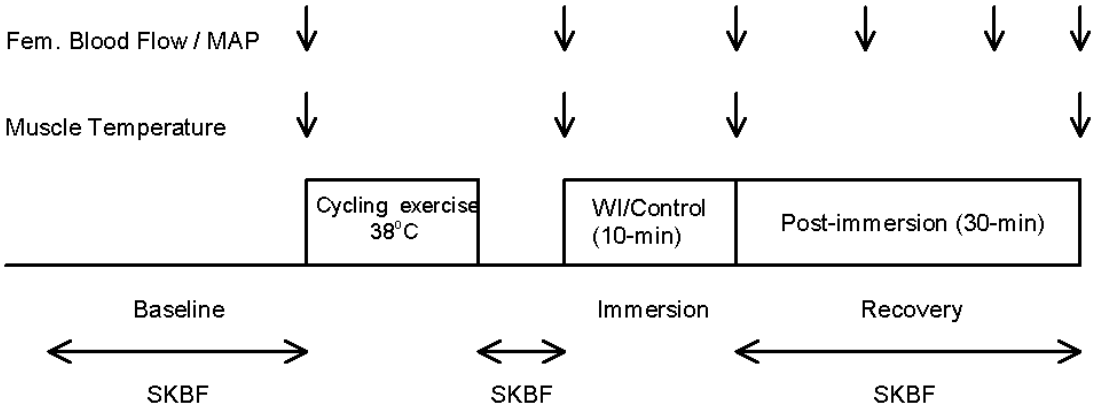

Figure 5.3.1. A schematic of the experimental design

5.3.4. Statistical analysis

It was estimated that a sample size of at least 12 participants would enable the detection of a 25 % reduction in FABF after 10-min of CWI, assuming a test-retest CV of 20 % and a statistical power of 80 % (G*Power, version 3.1, Dusseldorf, Germany). A two-factor (condition x time) within-participants general linear model (GLM) was used to evaluate treatment differences between the 8 °C, 22 °C and CON conditions. A three-way GLM (condition x depth x time) was used to analyse muscle temperature (SPSS version 18.0, Statistical Package for the Social Sciences, Chicago, IL). The assumption of sphericity (homogeneity of variance) was assessed and corrected for using the Greenhouse Geisser ε (Atkinson & Nevill, 2001). The main effect of condition was followed-up using multiple comparisons, as there were 3 levels for condition. A significant effect of time was followed up with planned multiple contrasts in line with the a priori hypotheses. Consequently, data at the specific time points were compared with the baseline (first) time point using Newman-Keuls multiple contrasts (Statistica, version 10, StatSoft Ltd, Milton Keynes, UK). As thigh and calf CVC was expressed as percentage change from pre-

immersion (zero), a one-sample t-test was used in follow-up analyses. Therefore, at each measured time-point, the main effect of time for thigh and calf CVC was compared with the pre-immersion (first) time point. Where a significant interaction between condition and time was observed, differences between conditions were examined at each time point using Newman-Keuls multiple contrasts. A one-way repeated measures GLM was used to determine any differences between conditions at baseline for FVC and at pre-immersion for thigh and CVC conductance. The α level for evaluation of statistical significance was set at $P < 0.05$.

5.4. RESULTS

5.4.1. Thermoregulatory responses

T_{rec} was similar between conditions at baseline (8 °C, 36.9±0.2; 22 °C, 36.9±0.2 °C; CON, 37.0±0.2 °C, $P > 0.05$) and prior to immersion (8 °C, 37.9±0.3 °C; 22 °C, 37.8±0.2 °C; CON, 37.9±0.2 °C, $P > 0.05$) and throughout the subsequent immersion and post immersion period ($P > 0.05$). T_{rec} reduced over time ($P < 0.01$) and to the greatest extent 30 min post-immersion (8 °C, 37.1±0.2 °C; 22 °C, 37.2±0.2 °C, CON, 37.1±1.0 °C, $P < 0.001$), but remained above baseline values ($P < 0.01$; Figure 5.4.1).

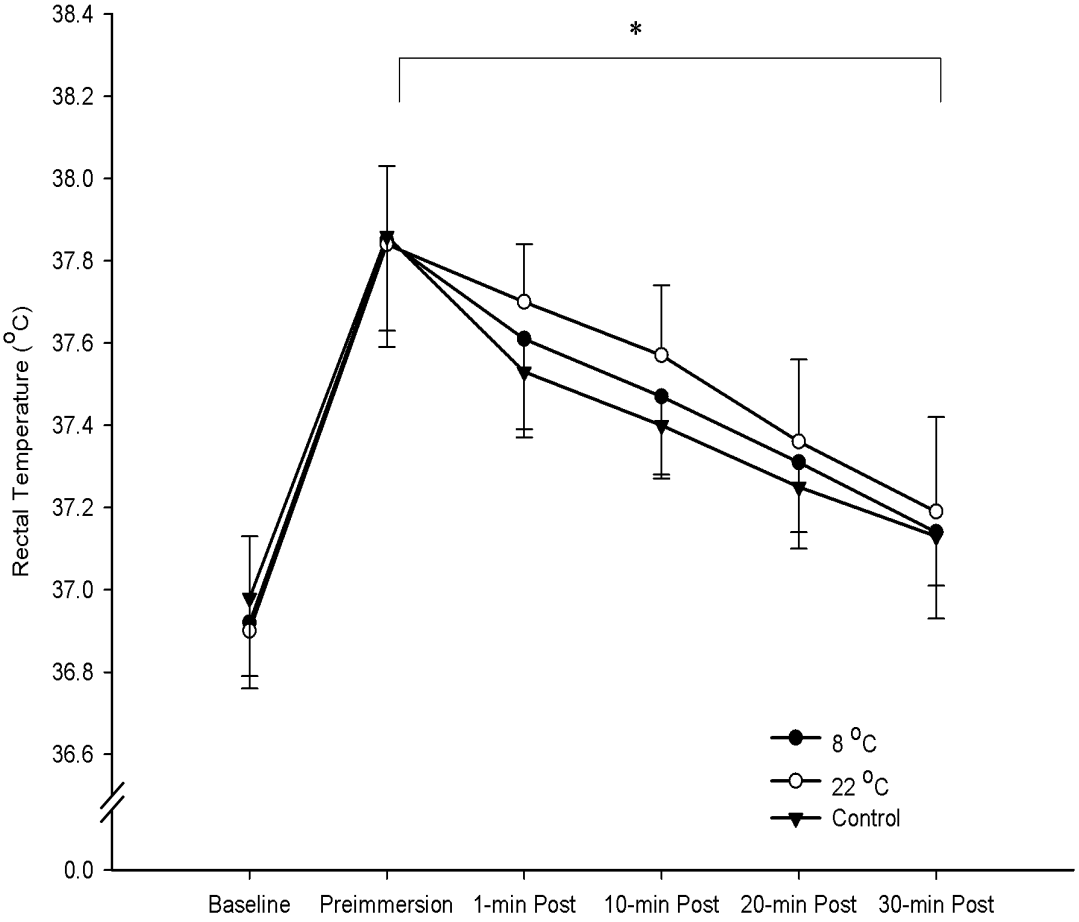

Figure 5.4.1. Rectal temperature pre and post immersion in 8 °C, 22 °C and CON (n = 11, mean ± SD). A main effect for time ($P < 0.01$) was found for rectal temperature. * Significant difference from baseline in each condition ($P < 0.05$).

Tsk_{mean} and Tsk_{thigh} were similar at baseline ($P > 0.05$) and before immersion ($Tsk_{mean;}$ 8 °C, 34.2±0.5 °C; 22 °C, 33.9±0.7 °C; CON, 34.1±0.8 °C, $P > 0.05$; $Tsk_{thigh;}$ 8 °C, 34.6±0.7 °C; 22 °C, 34.0±1.0 °C; CON, 34.6±0.9 °C, $P > 0.05$), but was lower throughout the post immersion period in the 8 °C and 22 °C conditions compared with CON ($P < 0.001$**)** (Figure 5.4.2). The colder water temperature also reduced Tsk_{mean} and Tsk_{thigh} to a greater extent compared to 22 °C at each time point during the post immersion period ($P < 0.001$). The rate of decrease in skin temperature was different between all conditions ($P < 0.001$) with the largest difference occurring immediately post immersion ($Tsk_{mean;}$ 8 °C, 25.5±1.2 °C; 22 °C, 29.5±1.2 °C; CON, 33.2±0.6 °C, *P*

< 0.001; $Tsk_{thigh;}$ 8 °C, 19.7±2.6 °C; 22 °C, 26.1±0.9 °C; CON, 34.1±0.6 °C, P < 0.001). Tsk_{mean} and Tsk_{thigh} increased during the 30-min recovery period in both cooling conditions whilst values remained relatively stable in CON. Tsk_{mean} and Tsk_{thigh} remained above baseline at the end of the recovery period in each condition ($Tsk_{mean;}$ 8 °C, 29.7±1.0 °C; 22 °C, 31.6±0.9 °C; CON, 33.8±0.8 °C, P < 0.01; $Tsk_{thigh;}$ 8 °C, 28.3±1.2 °C; 22 °C, 29.3±0.7 °C; CON, 33.6±1.1 °C, P < 0.001).

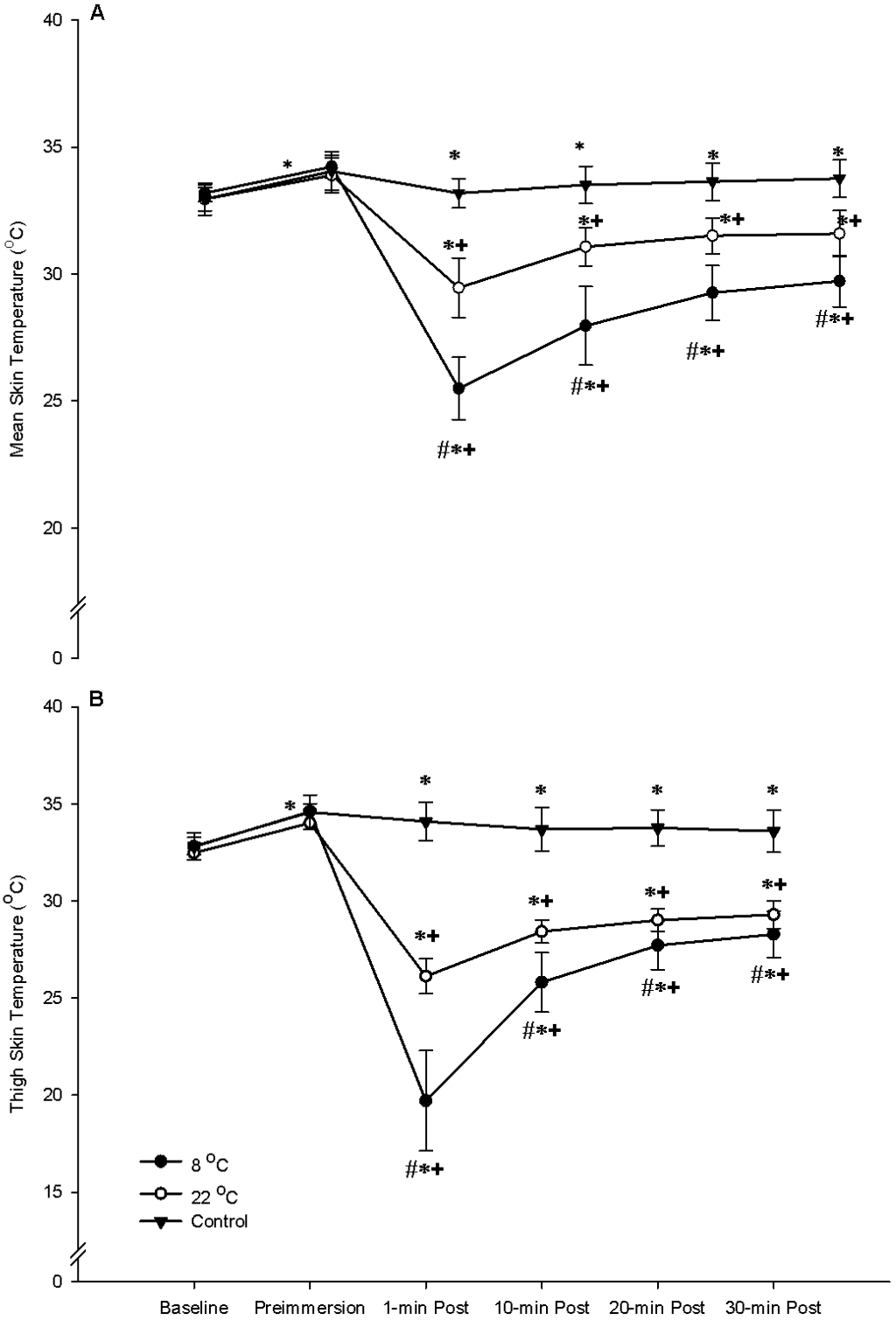

Figure 5.4.2. Mean skin temperature (A) and thigh skin temperature (B) pre and post-immersion in 8 °C, 22 °C and CON (n = 12, mean ± SD). Main effects for condition ($P < 0.001$) and time ($P < 0.001$), along with a significant interaction between condition and time ($P < 0.001$) were found for Tsk_{mean} Tsk_{thigh}. * Significant difference from baseline ($P < 0.05$). + Significant difference vs CON ($P < 0.05$). # Significant difference between cooling conditions $P < 0.05$).

T_{muscle} at a depth of 3cm was similar between conditions prior to immersion (8 °C, 37.9±0.3 °C; 22 °C, 38.0±0.2 °C; CON, 38.0±0.4 °C, $P > 0.05$), 2 cm (8 °C, 37.6±0.3 °C; 22 °C, 37.7±0.3 °C; CON, 37.6±0.6 °C, $P > 0.05$), and 1 cm (8 °C, 37.1±0.5 °C; 22 °C, 37.2±0.4 °C; CON, 37.1±0.7 °C, $P > 0.05$). T_{muscle} was reduced over time ($P < 0.01$) (Figure 5.4), generally decreasing immediately after immersion ($P < 0.01$) and continuing to decrease up to 30 min post immersion ($P < 0.01$). These reductions depended on probe depth ($P < 0.01$). At the deeper depths, the greatest declines in T_{muscle} occurred 30 min post immersion ($P < 0.01$). At the 1 cm probe depth, the greatest decline in T_{muscle} was observed immediately after immersion in the cooling conditions, before gradually increasing towards baseline values at the end of the recovery period (Figure 5.4.3).

The reductions in T_{muscle} over time were also dependent on condition ($P < 0.001$). T_{muscle} was generally lower in the cooling conditions both immediately (8 °C, 34.06±0.29 °C; 22 °C, 35.57±0.23 °C; CON, 36.83±0.14 °C, $P < 0.001$) and 30 min following immersion (8 °C, 34.51±0.22 °C; 22 °C, 35.21±0.21 °C; CON, 36.50±0.11 °C, $P < 0.001$). At both time points, there was also a difference between cooling conditions ($P < 0.001$), with a greater decrease in T_{muscle} observed in colder water ($P < 0.001$).

The differences in T_{muscle} between condition and time points were in turn dependent on the probe depth ($P < 0.001$). Compared to CON, both cooling conditions induced marked reductions in T_{muslce} immediately following immersion at 1 cm and 2 cm probe depths ($P < 0.001$). T_{muscle} was also reduced at a 3 cm probe depth in the 8 °C condition ($P < 0.001$), but not 22 °C ($P = 0.16$; Figure 5.4.3). At 30 min post

immersion, T_{muscle} was also lower in both cooling conditions compared to CON across all probe depths ($P < 0.01$). T_{muscle} was also reduced in 8 °C cooling compared with 22 °C immediately and 30 min following immersion at 1 cm ($P < 0.001$) and 2 cm ($P < 0.001$) probe depths. T_{muscle} was similar at the 3 cm probe depth immediately following immersion ($P = 0.16$), however, it showed a tendency (i.e. < 1.0) to be lower in 8 °C at 30 min post-immersion ($P = 0.06$; Figure 5.4.3).

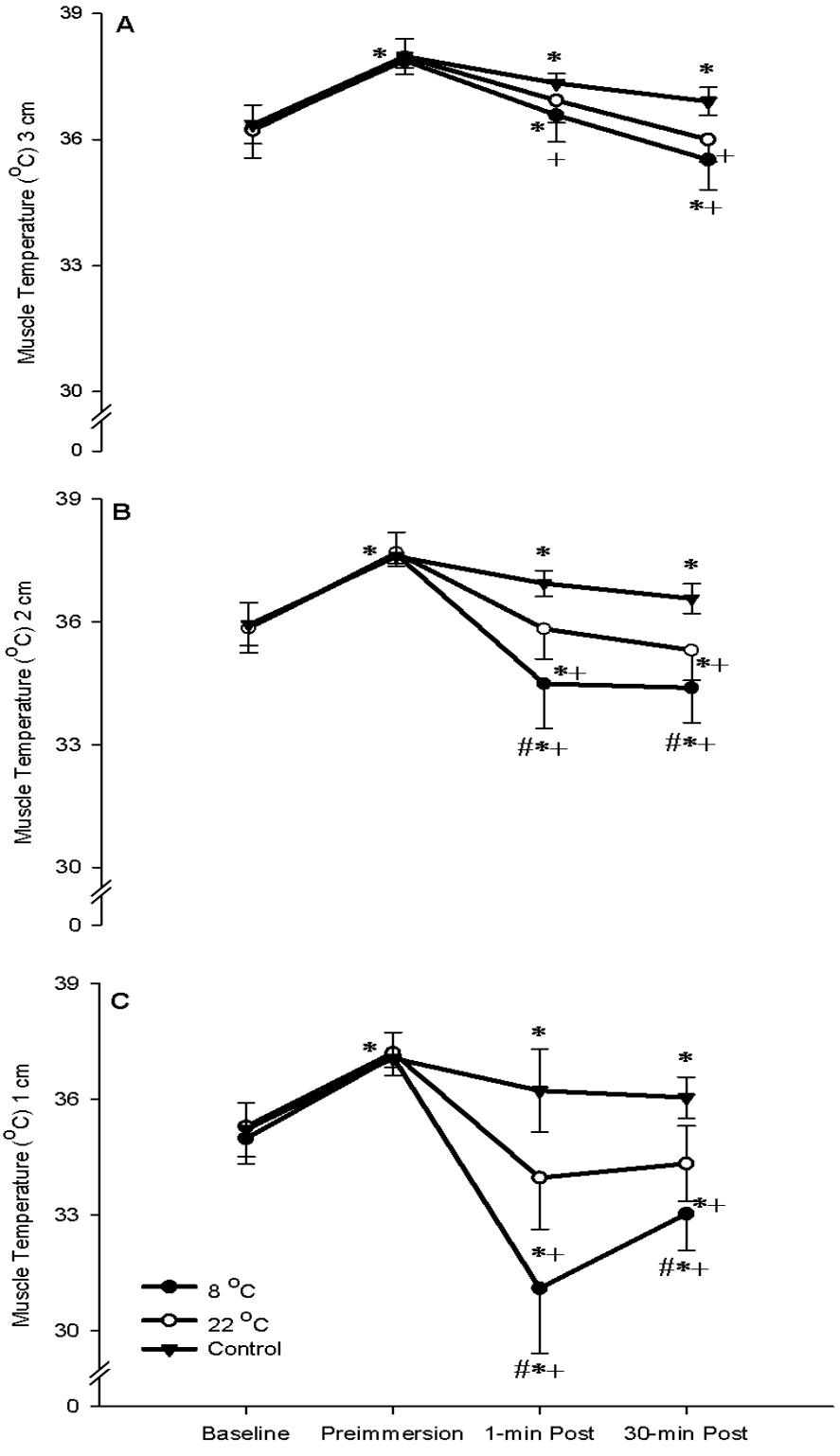

Figure 5.4.3. Muscle temperature pre and post immersion, at temperature probe depths of 3 cm (A), 2 cm (B), and 1 cm (C) (n =12, mean ± SD). Main effects for condition (P < 0.01) and time (P < 0.01) were found along with a significant interaction among condition, time and probe depth (P < 0.01). *Significant difference from baseline (P < 0.05). +Significant difference vs CON (P < 0.05). #Significant difference between cooling conditions (P < 0.05).

5.4.2. Heart rate and mean arterial pressure responses

Mean HR across the immersion and post immersion period was similar between conditions ($P = 0.17$), however the change in HR was different ($P < 0.001$). HR was similar immediately prior to immersion (8 °C, 88±2 beats·min^{-1}; 22 °C, 86±2 beats·min^{-1}; CON, 87±3 beats·min^{-1}, $P > 0.05$), but increased slightly during the 10 min immersion in 8 °C water (91±2 beats·min^{-1}) compared with 22 °C (78±3 beats·min^{-1}; $P < 0.001$) and CON (80±2 beats·min^{-1}; $P < 0.001$). HR remained similar between all conditions until the end of the post immersion recovery period ($P > 0.05$) and remained above baseline at the end of the 30 min recovery period in each condition ($P < 0.001$; Figure 5.4.4).

MAP was generally higher in the 8 °C condition (83±2 mmHg) compared with 22 °C (79±2 mmHg) and CON (79±2 mmHg) conditions ($P < 0.01$). The change in MAP over time was also different between conditions ($P < 0.01$). MAP was similar immediately pre immersion ($P > 0.05$), however a higher MAP was observed during the 10 min immersion and initial 10 min post immersion period in 8 °C water (86±10 mmHg) compared to 22 °C (79±7 mmHg) and CON (78±7 mmHg) conditions ($P < 0.05$). MAP was similar between all conditions throughout the remaining period of the post immersion phase ($P > 0.05$) and remained below baseline at the end of the 30-min recovery period in the 22 °C and CON conditions ($P < 0.01$) but similar to baseline in the 8 °C condition ($P = 0.9$; Figure 5.4.4).

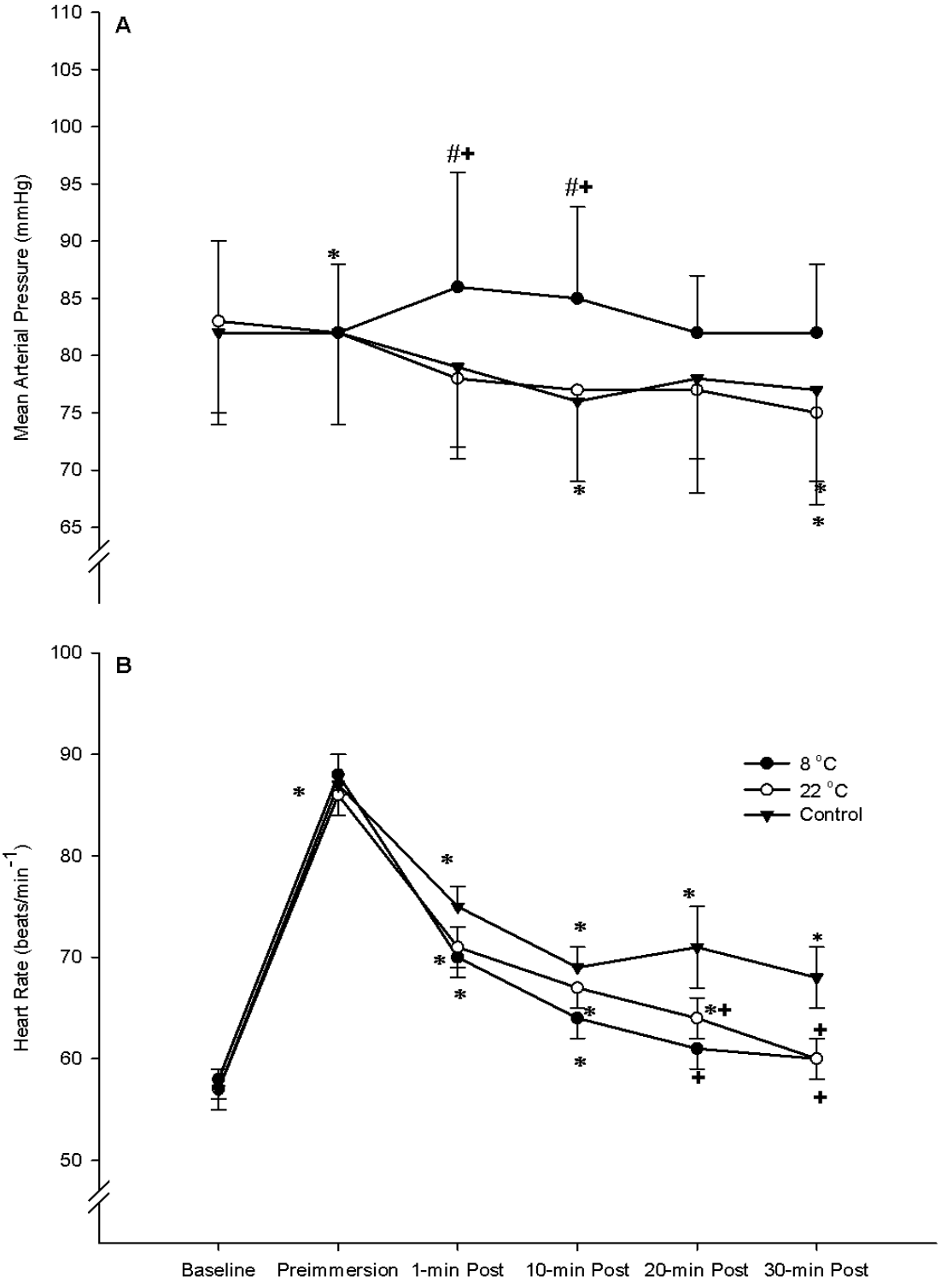

Figure 5.4.4. Mean arterial pressure (n = 12), (A) and heart rate (n = 11), (B) pre and post immersion in 8 °C, 22 °C and CON (mean ± SD). A main effect of time was found for both MAP and HR (P < 0.001). Main effects for condition (P = 0.01) and interaction (P = 0.02) were also found for MAP. *Significant difference from baseline (P < 0.05). + Significant difference vs CON (P < 0.05). #Significant difference between cooling conditions (P < 0.05).

5.4.3. Femoral artery blood flow responses

FABF (P = 0.66) and FVC (P = 0.37) were similar between cooling conditions but lower compared to CON (P < 0.01). The rate of decrease in FABF (P = 0.02) and conductance (P = 0.04) was greater in both cooling conditions in contrast to CON. FABF and FVC was similar immediately pre immersion (P > 0.05), however a lower FABF and FVC (~40 %) was observed from 10-min post immersion until the end of the 30-min recovery period (P < 0.05; Figure 5.4.5). At the end of the 30 min recovery period, FABF and FVC (~55 %) were below CON values (P < 0.01). FABF and FVC were reduced by ~30 % and ~75 % relative to baseline and pre-immersion values respectively as a consequence of cooling.

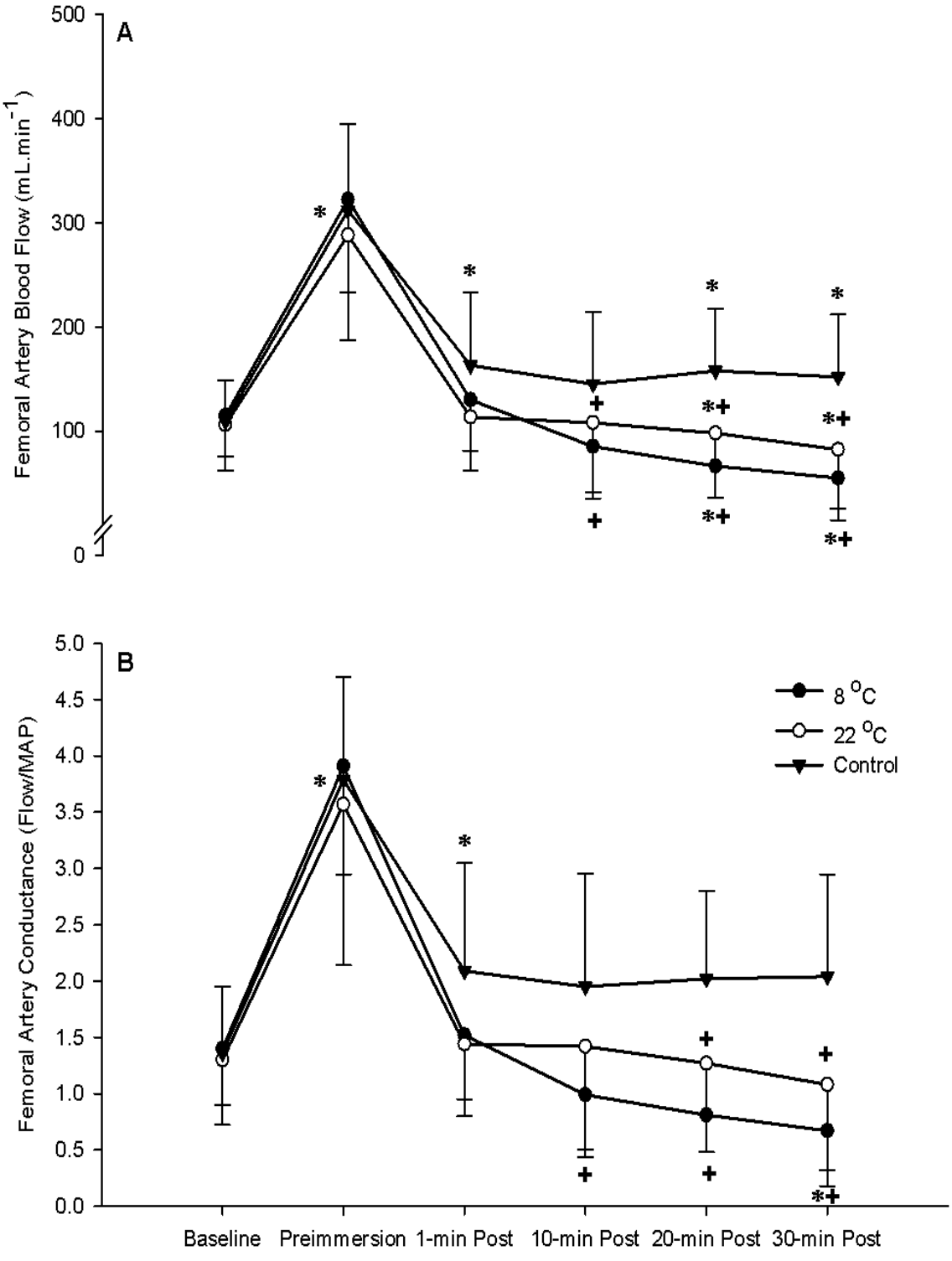

Figure 5.4.5. Femoral artery blood flow (A) and conductance (B) pre and post immersion in 8 °C, 22 °C and CON (n = 12, mean ± SD). A main effect for condition ($P < 0.01$) and time ($P < 0.01$) was found for both artery flow and conductance. There was also a significant interaction between condition and time for both artery flow ($P = 0.01$) and conductance ($P = 0.02$). * Significant difference from baseline ($P < 0.05$). + Significant difference vs CON ($P < 0.05$).

5.4.4. Cutaneous blood flow responses

Pre-immersion thigh (8 °C, 50.2±8.3 AU; 22 °C, 46.2±5.2 AU; CON, 51.4±6.1 AU, P = 0.74) and calf (8 °C, 30.2±2.7 AU; 22 °C, 32.0±4.2 AU; CON, 28.8±3.9 AU, P = 0.64) CVC was similar between conditions**.** When the data were expressed as a percentage change from pre-immersion, there was greater skin vasoconstriction observed in both cooling conditions at the thigh ($P < 0.01$) and calf ($P < 0.05$) relative to CON during immersion and throughout the post-immersion recovery period (Figure 5.4.6). No difference was observed between cooling conditions ($P > 0.05$). Significant vasoconstriction relative to pre-immersion was observed throughout the post-immersion period under both cooling conditions (~60-70 %).

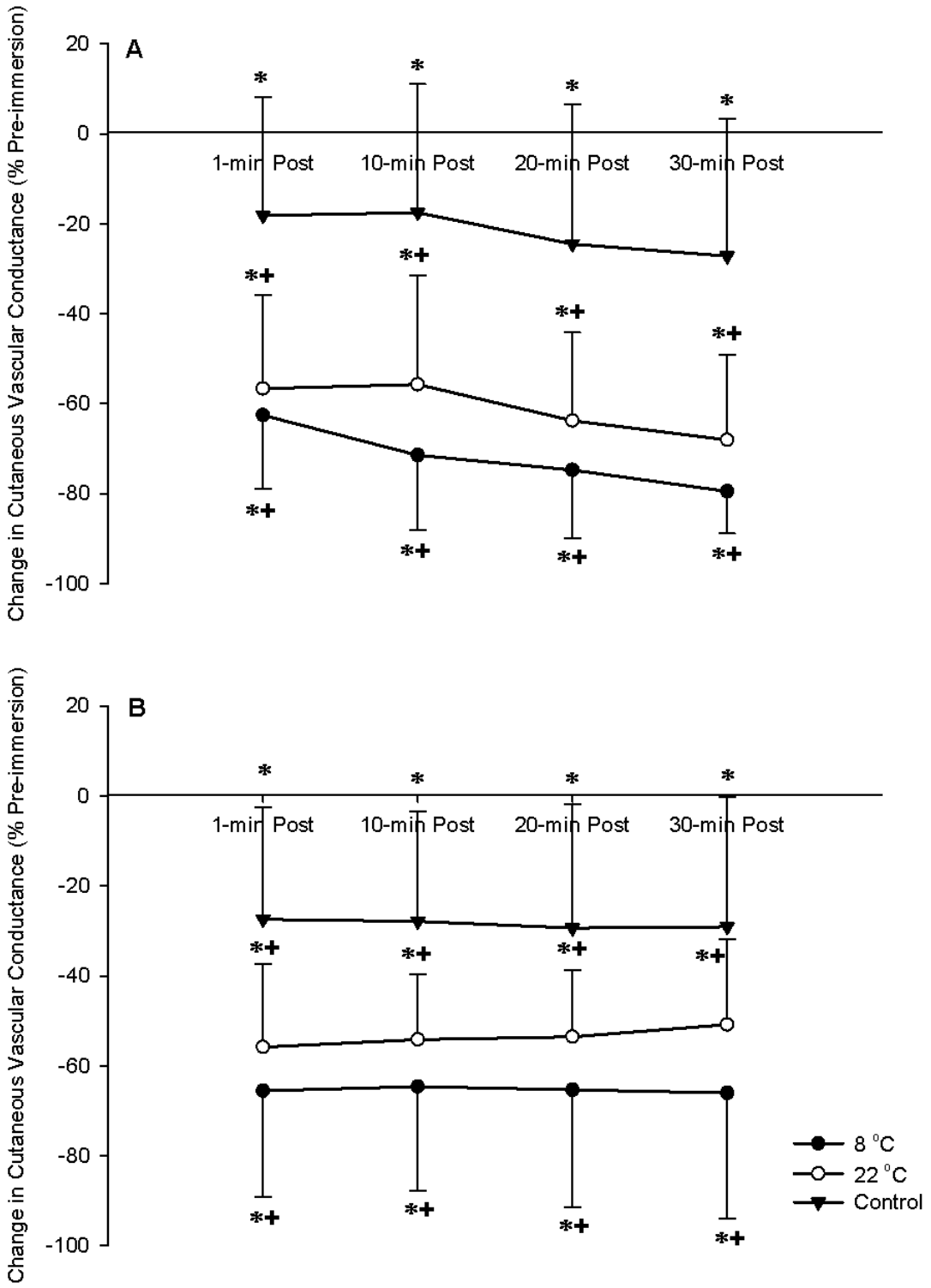

Figure 5.4.6. Percentage change in thigh cutaneous vascular conductance (A) and calf vascular conductance (B) from pre immersion in 8 °C, 22 °C and CON (n =12, mean ± SD). Main effects for condition (P < 0.01) were found for both thigh and calf cutaneous vascular conductance. A main effect for time (P = 0.01) was also found for thigh conductance. * Significant difference from Pre immersion (P < 0.05). + Significant difference vs CON (P < 0.05).

5.5. DISCUSSION

The major finding of this study is that 10 min of immersion in both 8 °C and 22 °C water after moderately intense endurance exercise reduces FABF (~55 %) and CBF compared with post exercise rest. Nevertheless, despite greater reductions in T_{muscle} and Tsk_{mean} and Tsk_{thigh} in colder water, no differences were observed between cooling conditions in terms of the magnitude of effect on FABF and CBF. Collectively, these findings suggest that the application of 8 °C and 22 °C water after exercise will influence any potential changes in muscle blood flow to a similar extent compared with post exercise rest. Furthermore, any additional treatment benefits arising from the use of colder temperatures are likely to be mediated through the effects of reduced tissue temperature rather than any further reductions in muscle blood flow. These findings provide important insights into the mechanisms, which underpin the use of CWI after exercise, i.e., reductions in tissue temperature and muscle blood flow, as well as a basis for improving treatment outcomes in clinical and sporting environments.

This is the first study, to our knowledge, that has addressed the influence of different degrees of cooling via whole limb CWI on changes in limb artery and CBF after exercise. Previous work has evaluated the influence of CWI on whole limb blood flow after exercise using VOP (Vaile *et al*., 2011). Although plethysmography can be reliably used to measure relative changes in whole limb blood flow at rest (Casey, Curry & Joyner, 2008), it does not distinguish between limb artery and skin blood flows and cannot be used during water immersion. Attempts to determine the effects of more localised cooling strategies such as ice packs on post exercise muscle blood flow have previously been undertaken using radioactive tracers (e.g. Xe^{133}; Thorsson

et al., 1985). However, estimation of muscle blood flow using radioactive tracers is associated with a number of recognized limitations (Rådegran, 1999) and ice packs are not ideal for cooling large muscle groups. Recent work (Ihsan *et al*., 2013; Roberts *et al*., 2015a) has examined the effects of CWI on relative changes in post exercise muscle perfusion using NIRS. However, NIRS only provides indirect estimates of relative changes in blood volume within the microcirculation. Furthermore, the NIRS signal is confounded under conditions were marked changes in skin blood flow arise (e.g. exercise, heating and cooling) (Davis *et al*., 2006). In the present study, changes in lower limb CBF were continuously measured using laser Doppler flowmetry while FABF was measured via conduit artery high-resolution duplex ultrasound (Gregson *et al*., 2011).

In the present investigation, 10 min of lower limb immersion in either 8 °C or 22 °C water reduced FVC by ~30 % and ~75 % relative to pre-exercise baseline and pre immersion values respectively. This led to a ~55 % reduction in FVC compared with CON at the end of the 30 min post exercise recovery period. The magnitude of the reduction in FVC with cooling relative to CON confirms observations in previous studies, which have utilized isotope clearance (Thorsson *et al*., 1985), and VOP (Vaile *et al*., 2011) measurement techniques to assess the effects of localized cooling (ice packs; Thorsson *et al*., 1985), and whole limb cooling (CWI; Vaile *et al*., 2011) on limb blood flow after exercise. In the present study, it is likely that activation of thermoreceptors in response to 8 °C cooling led to a reflex increase in sympathetic nerve activity and the reduction in FABF (Kellogg, 2006, Kregal, Seals & Callister, 1992). Indeed this reduction in blood flow and the accompanying increases in HR and MAP in colder water are consistent with the cardiovascular cold pressor response

(Lovallo, 1975; Raven *et al*., 1975; Wray *et al*., 2007). Despite a similar reduction in FABF in 22 °C water, no marked changes in HR or MAP were observed. Similar findings, at comparable water temperatures, have previously been reported (Gregson *et al*., 2011), with reductions in blood flow in temperate water attributed to activation of non-noxious thermoreceptors (Kregal, Seals & Callister, 1992, Wilson *et al*., 2007) known to be operable within the skin temperatures seen in this study (Hensel & Bowman, 1960).

In line with the changes in FABF, a similar reduction in CBF was observed under both cooling conditions compared with the CON. Taken together, the similar change in FABF and CBF suggest that both cooling strategies will be equally effective in promoting any potential reductions in muscle blood flow that may occur when applied immediately after exercise. The present changes in CBF contrast with previous work under resting conditions, where cold induced vasodilation led to higher CBF in 8 °C water compared to 22 °C (Gregson *et al*., 2011). Under resting conditions, increases in CBF during colder water immersion may redistribute blood from the underlying muscle, consequently colder water may be more effective in reducing muscle blood flow and inflammation at rest by virtue of its associated increase in CBF (Gregson *et al*., 2011). Differences in the cutaneous responses to marked cooling (8 °C) after exercise compared with rest may reflect the exercise-induced increase in body and local limb temperatures, which preceded immersion. In nonacral (hairy) skin, the precise mechanisms mediating cold-induced vasodilation have yet to be elucidated, however, marked local cooling may directly inhibit the normal vasoconstrictor response leading to vasodilation (Faber, 1988). Increases in skin temperature and/or core temperature associated with heat stress attenuate cutaneous vasoconstrictor

responsiveness (Wilson, Cui & Crandall, 2002). Consequently, the increased body temperature (and attendant skin blood flow) before immersion in the present investigation may have reduced the degree of vasoconstriction and prevented skin blood flow reaching low levels and the associated onset of cold-induced vasodilation, leading to sustained vasoconstriction in both 8 °C and 22 °C conditions. This sympatholytic effect may also account for the similar CBF observed in the two cooling conditions after immersion despite a lower skin temperature in the 8 °C condition. Indeed, a local cooling stimulus of similar magnitude to the difference in skin temperature between conditions (~6 °C) during the initial 10 min following immersion has been shown to induce greater reductions in skin blood flow when occurring at rest compared to those presently observed (Hodges *et al*., 2006).

The physiological effects of cryotherapy are thought to be mediated through temperature-induced reductions in microvascular blood flow around the injured area, which in turn reduces edema and the induction of inflammatory events (Lee *et al*., 2005, Thorlacius *et al*., 1998). Whole limb CWI may therefore be an effective treatment modality by virtue of its effect on T_{muscle}. Our findings indicate that both 8 °C and 22 °C water decreased deep and superficial T_{muscle} relative to the CON, with greater reductions in colder water. Furthermore, greater reductions in superficial temperature were initially observed after immersion with deep T_{muscle} declining to a greater extent 30 min after immersion. The observation of a sustained reduction in deep tissue temperature and blood flow to the deeper muscle tissue confirm previous reports at rest (Gregson *et al*., 2011) and provide support for the application of CWI soon after cessation of the activity that has led to EIMD and injury. Similarly, the transition from superficial to deep tissue cooling confirms findings from previous

studies undertaken at rest, which have employed localized cooling (Enwemeka *et al*., 2002) and whole limb immersion cooling (Gregson *et al*., 2011, Johnson *et al*., 1979). The increase in skin temperature and superficial (1 cm depth) T_{muscle} during the 30 min after water immersion partly reflects removal of the cooling source *per se* and subsequent exposure of the limbs to room air temperature. In addition, hemodynamic exchange between the cooler surface and the warmer deeper tissue is likely to have promoted a net flow of heat leading to a gradual increase in superficial tissue temperature and a corresponding decrease in deep tissue temperature over time (Enwemeka *et al., 2002*). The greater decrease in Tsk_{thigh} and T_{muscle} in the colder water may provide an analgesic response, which could help alleviate the pain associated with DOMS and muscle injury.

Recent work demonstrated that marked increases (~4 °C) in deep (~2 cm) T_{muscle} associated with local heating, induced increases in skeletal muscle blood flow (Heinonen *et al*., 2011). As noted previously, FABF in the present investigation was reduced with cooling relative to the CON; however, similar reductions in blood flow were observed in the cooling conditions despite differences in T_{muscle}. In contrast to skin, where an elevated body temperature impairs vasoconstrictor responsiveness to cooling, such effects do not arise in the whole limb (Keller *et al*., 2010) and therefore do not explain the failure to observe greater reductions in FABF with greater decreases in T_{muscle} in 8 °C water relative to 22 °C. Similar reductions in FABF in response to 8 and 22 °C cooling have previously been reported at rest (Gregson *et al*., 2011). It is therefore possible that the magnitude of the difference in deep T_{muscle} observed between cooling conditions (~0.5 °C) in the present investigation may not have been sufficient to directly modify FABF.

The present findings have shown colder water temperatures cause greater reductions in muscle tissue temperature, supporting the view that better treatment outcomes following muscle injury may arise from the selection of cooling modalities, which promote greater tissue cooling (Merrick, Jutte & Smith, 2003). Similar to CWI under resting conditions, a decreased pain tolerance (Gregson *et al*., 2011, Wolf & Hardy, 1941) was observed in several participants during the 8 °C noxious cooling, especially in the extremities. Although T_{muscle} was not reduced to the same extent as with 8 °C cooling, deep muscle temperature was still significantly reduced following 22 °C water immersion compared with the CON. Less noxious cooling above temperatures which cause cold pain (> 18 °C; Wolf & Hardy, 1941) may therefore provide a suitable alternative to individuals unable to tolerate more noxious degrees of cooling (Gregson *et al*., 2011). However, it remains to be ascertained whether colder water temperatures provide any added benefit to treatment outcomes than less noxious cooling in athletic settings and should provide a focus for future work.

5.6. SUMMARY

Post exercise FABF and CBF were assessed during and post water immersion at 8 °C or 22 °C compared to rest. The present findings suggest that being immersed in cool and cold-water temperatures causes similar reductions in blood flow. However, greater reductions in T_{muscle} arise with colder water temperatures suggesting that such modalities may be more effective in the treatment of EIMD and injury. These findings provide important insights into the possible mechanisms responsible for CWI in alleviating inflammation in sport and athletic contexts.

CHAPTER 6

INFLUENCE OF COLD-WATER IMMERSION ON LIMB AND CUTANEOUS BLOOD FLOW AFTER RESISTANCE EXERCISE

6.1 INTRODUCTION

Lower limb CWI is a widely used recovery method to reduce the negative symptoms associated with high-intensity or unaccustomed exercise (Bailey *et al*., 2007; Leeder *et al*., 2012). Cooling of the exercised muscles is proposed to attenuate the acute inflammatory response, thereby reducing the development of EIMD and decrements in muscular function and soreness (Smith, 1991). Previous studies have shown that CWI decreases limb muscle temperature and blood flow when applied at rest (Gregson *et al*., 2011) and following continuous endurance exercise such as cycling (see Chapter 5; Vaile *et al*., 2011) and treadmill running (Ihsan *et al*., 2013). Taken together, these data suggest that CWI, especially at colder temperatures, may provide better recovery from endurance type exercise.

Previous studies have examined the impact of CWI immediately following resistance type exercise and found improvements in muscle function and strength when compared to active recovery (Roberts *et al*., 2015a). The strength improvements with CWI were modulated by T_{muscle} and potentially blood flow (muscle oxygenation) when compared to active recovery (Roberts *et al*., 2015a). No study, to date has directly examined the impact of CWI on limb blood flow following a bout of resistance exercise. This is important to establish, since resistance exercise can cause a different haemodynamic, thermoregulatory and mechanical stress compared with endurance exercise. For example, the magnitude of the local limb blood flow increase is lower during a bout of resistance exercise relative to endurance exercise (e.g. cycling), similarly, the intermittent nature and potential for breath holding in resistance exercise contrasts the linear increase and plateau in limb blood flow in endurance exercise (MacDougall *et al*., 1992; Mortensen *et al*., 2008). It is also

possible that resistance exercise does not cause increases in core body temperature of the same magnitude as endurance exercise (Deschenes *et al*., 1998). Moreover, resistance exercise stimulates greater muscle damage compared with other modes of exercise, such as cycling and running (Dolezal *et al*., 2000; Howatson *et al*., 2012).

In Chapter 5, it was shown that CWI at different water temperatures similarly decreases post-cycling lower limb blood flow despite greater reductions in T_{muscle} and Tsk_{thigh} temperatures in colder water. It is currently unknown if the differences in hemodynamic and temperature responses mediated by resistance, relative to endurance, exercise, would impact upon post-resistance exercise responses to CWI of various temperatures.

6.2 PURPOSE

The aim of this study was to examine the effects of cold (8 ºC) and cool (22 ºC) water immersion on lower limb blood flow and muscle temperature changes, after a typical bout of resistance exercise. It was hypothesized that whole-limb immersion in 8 ºC and 22 ºC water would decrease femoral artery and skin blood flow to a similar extent after resistance exercise, but colder water would reduce muscle temperature to a greater extent.

6.3 MATERIALS AND METHODS

6.3.1 Participants

Twelve recreationally active men were studied (mean±SD: age, 26±6 yrs; height, 1.8±0.1 m; mass, 77.5±11.2 kg; BSA, 1.9±0.2 m^2). The participants typically performed resistance exercise at least three times per week, which included Olympic

barbell squatting exercise at least once per week (self-report questionnaire). Participants completed all the pretest criteria prior to testing (see section 3.1.2, 3.1.3 and 3.1.4).

6.3.2 Preliminary testing

Two weeks prior to the commencement of the experimental trials, each participant completed a 10-repetition maximum (10 RM) parallel squat assessment using a Smith machine. The squat protocol consisted of a warm up set, using only the bar, followed by progressive increases in load until the attainment of the 10 RM within five attempts (Baechle & Earle, 2000). The following week, participants completed 4 sets of the predetermined 10 RM squat exercise interspersed with 2 min rest periods. This was performed to reduce the magnitude of muscle damage and the subsequent secondary inflammatory cascade from the exercise stimulus, which might influence blood flow. In addition, a preconditioning stimulus confers a protective repeated bout effect (Howatson & van Someren, 2008). This reduces the potential for any order effect, because all participants would be familiar with the protocol provided by the pre-conditioning stimulus, which has been shown to confer this protective effect (Chen *et al*., 2012).

6.3.3 Experimental protocol

The experimental trials were performed in a randomized counterbalanced order, at least 7-days following the second familiarization session and at least 7-days apart. Each participant was required to complete 4 sets of 10 RM squats followed by a 10 min period of immersion in either 8 °C or 22 °C water or seated rest (CON; see Figure 6.3.1). The water temperatures and immersion protocol was based on previous

studies (Gregson et al., 2011; Wilcock, Cronin & Hing, 2006). On arrival at the laboratory, the participant's self-inserted a rectal probe and a HR monitor was positioned across the chest. Participants were then laid in a supine position for 30 min on a bed (next to the water tank) for the attachment of instrumentation and to stabilize physiological status (Olive, McCully & Dudley 2002), wearing training shorts. Following baseline measurements (10 min), participants completed 4 sets of 10 RM squats interspersed with a 2 min rest period between sets. Participants then returned to the supine position for 10 min for post-exercise/pre-immersion measurements. Participants were then raised from the bed in a semi-recline position using an electronic hoist and either lowered into the water tank (ECB, Gloucester, U.K.) for 10 min (see section 3.7), or remained suspended above the bed (CON). At the end of immersion, participants were returned to the bed using the electronic hoist and remained supine for 30 min.

T_{rec} (see section 3.2.1), $Tsk_{chest,}$ $Tsk_{forearm,}$ $Tsk_{thigh,}$ and Tsk_{calf} and Tsk_{mean} (see section 3.2.2), HR (see section 3.5.1) and thigh and calf CBF (see section 3.3) were continuously monitored at baseline, before immersion, and throughout the 30 min post-immersion period. T_{muscle} (see section 3.2.3) was recorded at baseline, immediately before and after immersion and 30 min after immersion. FABF (see section 3.4.1) and MAP (see section 3.5.2) were measured at baseline, before immersion, immediately after immersion, and at 10 min intervals throughout the 30 min post-immersion period. Perceived thermal comfort (see section 3.6.1) and shivering (see section 3.6.2) were also recorded at the same time points. All pre and post-immersion measurements were made in a supine position. A schematic illustration of the experimental design is shown in Figure 6.3.1.

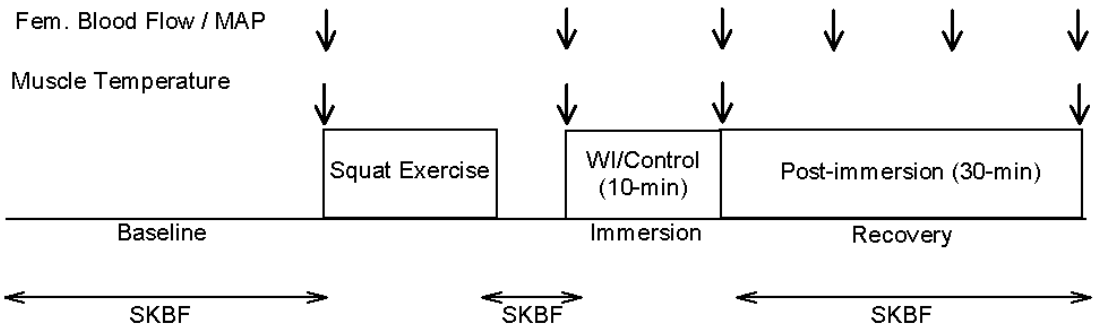

Figure 6.3.1. A schematic of the experimental design

6.3.4 Statistical Analysis

It was estimated that a sample size of at least 6 participants would have 90 % power to detect a 175 mL·min reduction in femoral artery blood flow following 10 min of cool (22 °C) water immersion, using an SD of the differences of 99 mL·min (Study 2) (G*Power, version 3.1, Dusseldorf, Germany). General linear modeling was used to evaluate treatment differences between conditions and over time (SPSS version 18.0, Statistical Package for the Social Sciences, Chicago, IL). Significant main effects and interactions were followed up using Newman–Keuls multiple comparisons (Statistica, version 10, StatSoft Ltd, Milton Keynes, UK). As thigh and calf CVC were expressed as percentage changes from pre immersion (zero), a one-sample t-test was used in follow-up analyses; each measured time-point was compared with pre-immersion (first time point). The α level for significance was set at $P<0.05$ and data are presented as mean±SD.

6.4 RESULTS

6.4.1.Thermoregulatory responses

T_{rec} was similar between conditions at baseline (8 °C; 37.0±0.2 °C; 22 °C; 37.0±0.3 °C; CON; 37.0±0.2 °C; $P > 0.05$). T_{rec} increased after exercise (8 °C; Δ 0.3±0.2 °C; 22 °C; Δ 0.2±0.1 °C; CON; 0.3±0.1 °C; $P < 0.001$) but remained similar between conditions immediately pre immersion (8 °C; 37.3±0.2 °C; 22 °C; 37.2±0.2 °C; CON; 37.2±0.2 °C; $P > 0.05$). T_{rec} decreased over the post immersion recovery period ($P < 0.001$), however, there was no difference between conditions at any time point ($P = 0.19$; Figure 6.4.1). T_{rec} returned to baseline values at the end of recovery period in the 22 °C (37.0±0.3 °C, $P = 0.71$) and CON (37.0±0.2 °C, $P = 0.95$) conditions but decreased slightly below baseline in 8 °C (36.9±0.3 °C, $P = 0.01$) (Figure 6.4.1).

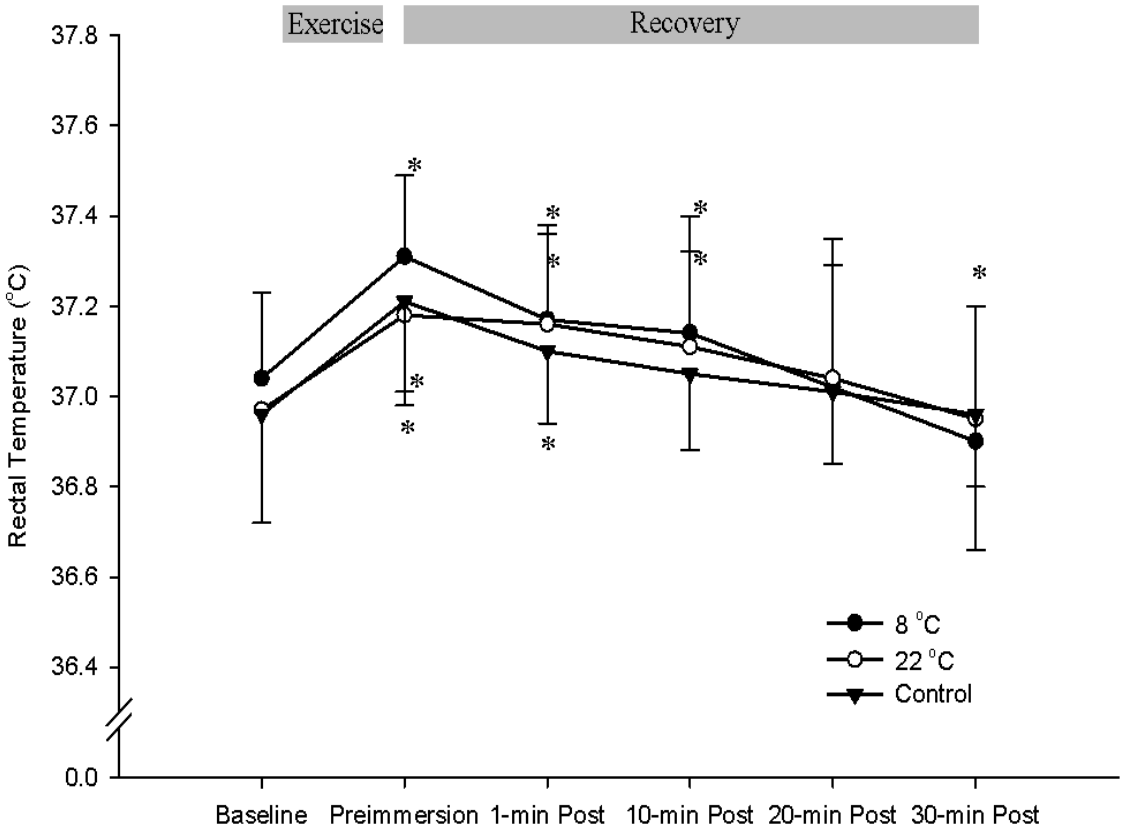

Figure 6.4.1. Rectal temperature pre and post immersion in 8 °C, 22 °C and CON (n = 12, mean ± SD). A main effect for time ($P < 0.001$) was found for rectal temperature. * Significant difference from baseline in each condition ($P < 0.05$).

At baseline, Tsk_{thigh} temperature was similar between conditions (8 °C; 33.2±0.7 °C; 22 °C; 32.4±1.0 °C; CON; 32.8±1.0 °C; $P > 0.05$), however Tsk_{mean} temperature was higher in the 8 °C condition compared with 22 °C and CON (8 °C; 33.8±0.4 °C; 22 °C; 33.1±0.8 °C; CON; 33.2±0.8 °C; $P < 0.01$). Tsk_{thigh} and Tsk_{mean} temperatures were similar between conditions after exercise (i.e. pre immersion) (Tsk_{mean}; 8 °C; 33.5±0.4 °C; 22 °C; 33.2±0.7 °C; CON; 33.2±0.7 °C; $P > 0.05$; Tsk_{thigh}; 8 °C; 33.5±0.6 °C; 22 °C; 33.2±0.9 °C; CON; 33.3±0.8 °C; $P > 0.05$), but was lower throughout the post immersion period in the 8 °C and 22 °C conditions compared with CON ($P < 0.001$) (Figure 6.4.2). The colder water temperature also reduced Tsk_{mean} and Tsk_{thigh} temperature to a greater extent compared to 22 °C at each time point

throughout the recovery period ($P < 0.01$). The rate of decrease in skin temperature was different between all conditions ($P < 0.001$) with the largest difference occurring immediately post immersion (Tsk_{mean}; 8 °C; 27.2±0.6 °C; 22 °C; 30.1±0.6 °C; CON; 33.3±0.7 °C; $P < 0.001$; Tsk_{thigh}; 8 °C; 18.4±1.4 °C; 22 °C; 26.4±1.2 °C; CON; 33.4±0.9 °C; $P < 0.001$). Tsk_{thigh} increased during the 30-min recovery period in both cooling conditions whilst values remained relatively stable in CON. Tsk_{thigh} and Tsk_{mean} temperatures remained below baseline at the end of the recovery period in the 8 °C (Tsk_{mean}; 30.7±0.6 °C; Tsk_{thigh}; 27.3±1.1 °C; $P < 0.001$) and 22 °C conditions (Tsk_{mean}; 31.3±0.7 °C; Tsk_{thigh}; 29.1±1.8 °C; $P < 0.001$) and unchanged in CON (Tsk_{mean}; 33.3±0.8 °C; Tsk_{thigh}; 33.1±0.9 °C; $P < 0.05$) (Figure 6.4.2).

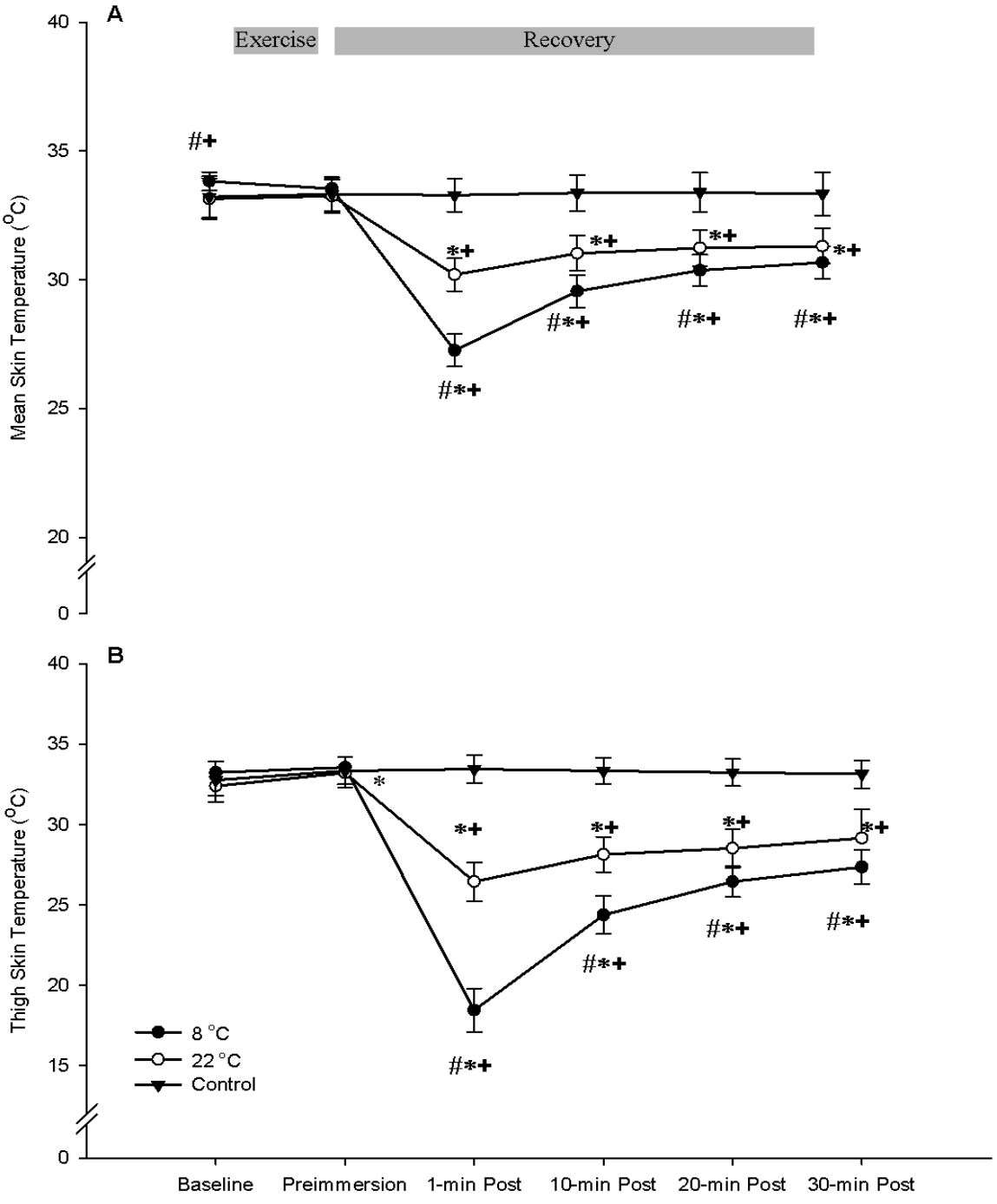

Figure 6.4.2. Mean skin temperature (A) (n = 11) and thigh skin temperature (B) (n =12) pre and post-immersion in 8 °C, 22 °C and CON (mean ± SD). Main effects for condition ($P < 0.001$) and time ($P < 0.001$), along with a significant interaction between condition and time ($P < 0.001$) were found for Tsk_{mean} and Tsk_{thigh}. * Significant difference from baseline ($P < 0.05$). + Significant difference vs CON ($P < 0.05$). # Significant difference between cooling conditions $P < 0.05$).

Baseline T_{muscle} was similar between conditions ($P > 0.05$) at 3 cm (8 °C; 36.2±0.3 °C; 22 °C; 35.9±0.5 °C; CON; 36.0±0.5 °C), 2 cm (8°C; 35.7±0.4 °C; 22 °C; 35.4±0.7 °C; CON; 35.5±0.7 °C), and 1 cm (8 °C; 34.9±0.6 °C; 22 °C; 34.8±0.8 °C; CON; 34.8±0.8 °C) depths. T_{muscle} was increased ($P < 0.001$) by the squat exercise protocol (i.e. pre immersion) at 3 cm (8 °C; 37.0±0.3 °C; 22 °C; 37.1±0.3 °C; CON; 37.0±0.2 °C), 2 cm (8 °C; 36.6±0.5 °C; 22 °C; 36.6±0.2 °C; CON; 36.6±0.2 °C), and 1 cm (8 °C; 35.9±0.9 °C; 22 °C; 36.0±0.3 °C; CON; 35.9±0.4 °C) depths, but remained similar between conditions ($P > 0.05$; Figure 6.4.3). T_{muscle} was reduced in both cooling conditions immediately after immersion compared with CON at 3 cm (8 °C, 34.6±0.9 °C; 22 °C, 35.8±0.5 °C; CON, 36.6±0.2 °C; $P < 0.001$), 2 cm; (8 °C, 32.0±1.4 °C; 22 °C, 34.1±0.9 °C; CON, 36.2±0.3 °C; $P < 0.001$) and 1 cm (8 °C, 29.5±1.5 °C; 22 °C, 32.6±0.8 °C; CON, 35.6±0.5 °C; $P < 0.001$) probe depths. T_{muscle} was also reduced in the cooling conditions compared with CON 30 min post immersion at 3 cm (8 °C, 33.5±0.9 °C; 22 °C, 34.8±0.4 °C; CON, 36.2±0.3 °C; $P < 0.001$; 2 cm (8 °C, 32.2±0.9 °C; 22 °C, 34.0±0.5 °C; CON, 35.8±0.3 °C; $P < 0.001$) and 1 cm (8 °C, 31±0.9 °C; 22 °C, 33.1±0.6 °C; CON, 35.2±0.5 °C; $P < 0.001$) depths. There was also a greater reduction in T_{muscle} at each depth in 8 °C cooling compared with 22 °C at both time points ($P < 0.001$; Figure 6.4.3).

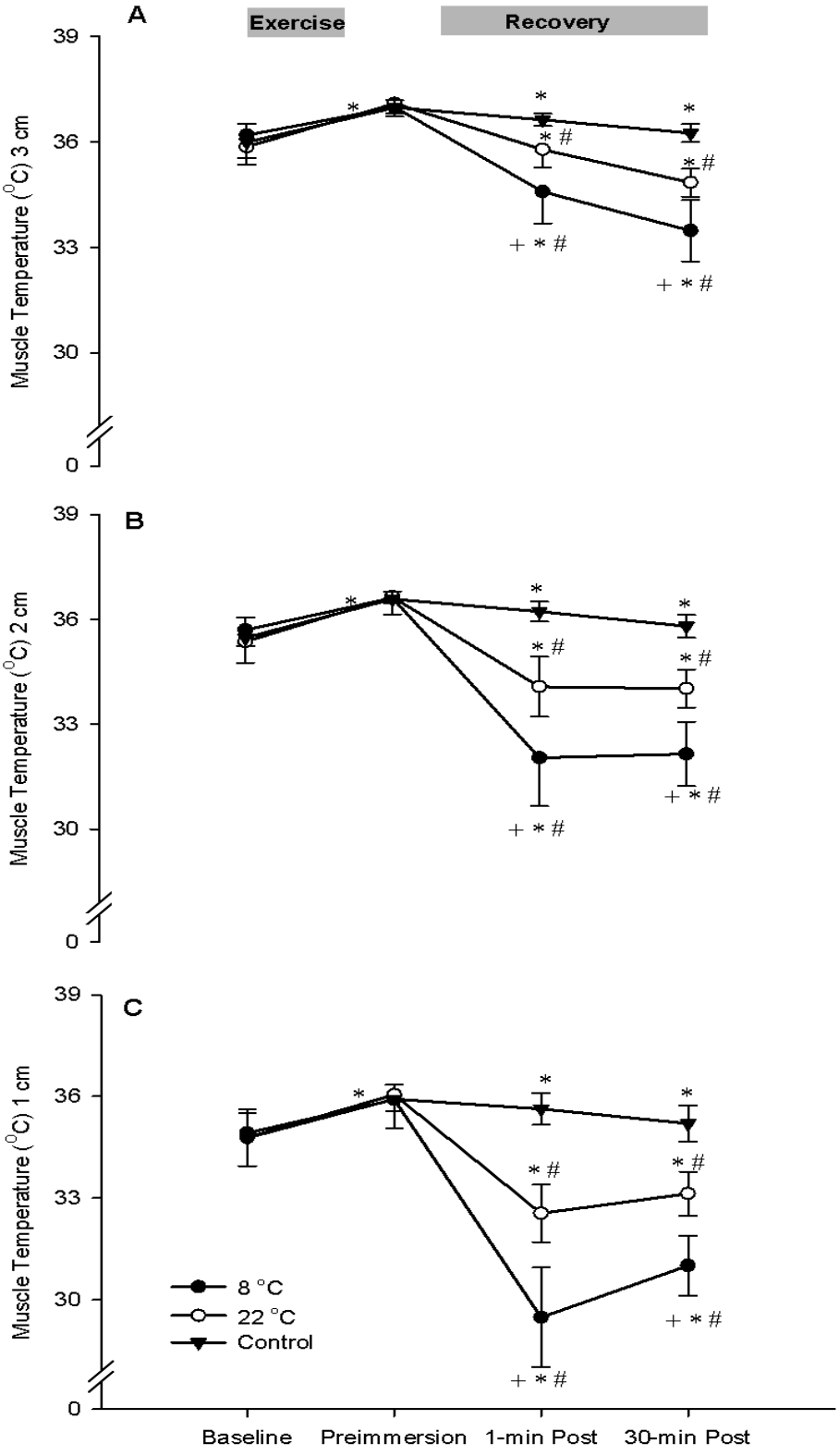

Figure 6.4.3. Muscle temperature pre and post immersion, at temperature probe depths of 3 cm (A), 2 cm (B), and 1 cm (C) (n =12, mean ± SD). Main effects for condition (P < 0.01) and time (P < 0.01) were found along with a significant interaction among condition, time and probe depth (P < 0.01). * Significant difference from baseline (P < 0.05). + Significant difference vs CON (P < 0.05). # Significant difference between cooling conditions (P < 0.05).

Thermal comfort was similar between conditions at baseline (8 °C, 5±1 AU; 22 °C, 5±1 AU; CON, 5±1 AU, $P > 0.05$) and immediately pre immersion (8 °C, 5±1 AU; 22 °C, 5±1 AU; CON, 5±1 AU, $P > 0.05$). Thermal comfort was lower after cooling; both immediately (8 °C, 2±1 AU; 22 °C, 3±1 AU; CON, 5±1 AU, $P < 0.001$) and 10 min post immersion (8 °C, 3±1 AU; 22 °C, 4±1 AU; CON, 5±1 AU, $P < 0.01$) compared with CON. A lower thermal comfort rating was also noted in the 8 °C condition, 20 min after immersion, compared to the CON condition (8 °C, 4±1 AU; CON, 5±1 AU, $P < 0.001$). Thermal comfort was also lower in the colder water compared with 22 °C for up to 10-min after immersion ($P < 0.001$). There was no difference in thermal comfort between conditions at the end of the 30 min recovery period (8 °C, 5±1 AU; 22 °C, 5±1 AU; CON, 5±1 AU, $P > 0.05$) with similar ratings to baseline. Slight to moderate shivering was observed during immersion in both cooling conditions compared with no shivering in CON. There was no shivering observed throughout the post immersion period under any experimental condition.

6.4.2 Heart rate, RPE and mean arterial pressure responses

HR was similar between conditions at baseline (8 °C, 65±9 beats·min^{-1}; 22 °C, 61±5 beats·min^{-1}; CON; 63±8 beats·min^{-1}; $P > 0.05$). Each set of 10 repetitions of squat exercise increased HR ($P < 0.01$; see Table 6.4.1) but values were not different between conditions ($P = 0.34$). HR remained above baseline values prior to immersion (8 °C; 77±11 beats·min^{-1}; 22 °C; 77±11 beats·min^{-1}; CON; 73±10 beats·min^{-1}; $P < 0.001$). RPE were also rated higher with each subsequent set of squat exercise ($P < 0.05$; see Table 6.4.1) with similar ratings recorded between conditions in each set ($P = 0.75$). There was no difference in pre-immersion HR between 22 °C and CON conditions ($P = 0.79$), however, a higher HR was recorded in the 8 °C

condition compared with the 22 °C ($P < 0.01$) and CON ($P < 0.01$) conditions, respectively. HR was slightly increased during colder water immersion (8 °C, 80±14 beats·min^{-1}; 22 °C, 69±9 beats·min^{-1}; CON; 71±7 beats·min^{-1}; $P < 0.001$), but remained similar between all conditions during the post immersion recovery period ($P > 0.05$) and returned towards baseline values ($P > 0.05$, Figure 6.4.4).

Table 6.4.1. Exercise responses

		Set 1	Set 2	Set 3	Set 4
8°C	HR	124±17	129±17**	131±18*	135±18*
	RPE	13±2	14±1*	14±2*	15±2*
22°C	HR	118±13	122±14**	127±15*	130±18*
	RPE	13±1	13±1***	14±1*	15±2*
Control	HR	116±16	123±17*	127±17*	131±17*
	RPE	13±1	14±2*	14±2*	15±2*

Values are means ± SD. HR, heart rate (beats·min^{-1}); RPE, ratings of perceived exertion (AU). A main effect for time (set) was found for HR ($P < 0.001$) and RPE ($P < 0.001$). Significant difference to Set 1 (*P < 0.001 ** P < 0.01 *** P < 0.05).

MAP was similar between conditions at baseline (8 °C; 84±9 mmHg; 22 °C; 86±5 mmHg; CON; 87±5 mmHg; $P > 0.05$) and pre immersion (8 °C; 89±5 mmHg; 22 °C; 88±5 mmHg; CON; 88±6 mmHg; $P > 0.05$). MAP was higher immediately post immersion in 8 °C water (95±7 mmHg) compared to 22 °C, (88±7 mmHg) and CON (87±4 mmHg) conditions ($P > 0.01$). MAP was similar between all conditions throughout the remaining period of the post immersion phase ($P > 0.05$). MAP returned towards baseline values at the end of the 30-min recovery period in the 22° C

and CON conditions ($P > 0.05$), but still remained elevated in the 8 °C condition (8 °C, 90±6; 22 °C, 90±5; CON, 89±7; $P = 0.02$; Figure 6.4.4).

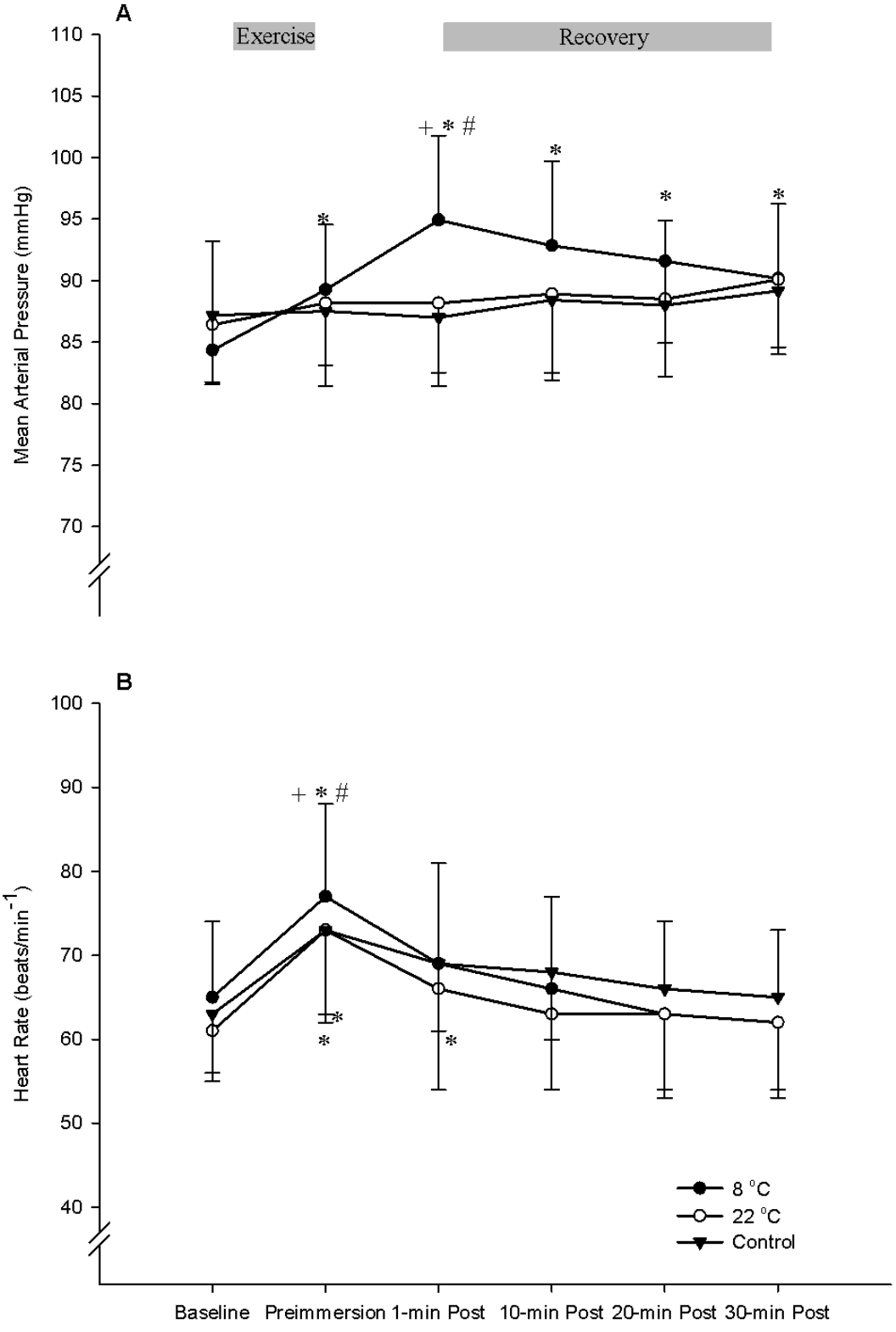

Figure 6.4.4. Mean arterial pressure (n = 12), (A) and heart rate (n = 12), (B) pre and post immersion in 8 °C, 22 °C and CON (mean ± SD). A main effect of time was found for both MAP ($P = 0.01$) and HR ($P < 0.001$). Main effects for interaction were also found for MAP ($P = 0.01$) and HR ($P = 0.001$). * Significant difference from baseline ($P < 0.05$). + Significant difference vs CON ($P < 0.05$). # Significant difference between cooling conditions ($P < 0.05$).

6.4.3. Femoral artery blood flow responses.

FABF (P = 0.65) and FVC (P = 0.77) were similar between cooling conditions but lower compared to CON (P < 0.01). The rate of decrease in FABF *(*P < 0.01) and FVC *(*P < 0.01) were also greater in both cooling conditions in contrast to CON. FABF and FVC were similar between conditions at baseline (P > 0.05). Exercise increased FABF and FVC by ~75 % and ~80 % respectively (P < 0.001), however there was no difference between conditions. A lower FABF and FVC (~50 %) was observed immediately post-immersion until the end of the 30-min recovery period in both cooling conditions compared with CON *(*P < 0.01; Figure 6.4.5). At the end of the 30-min recovery period, FABF and FVC (~55 %) remained below CON values (P < 0.01). Cooling reduced FABF and FVC by ~60 % and ~75 % relative to baseline and pre-immersion values, respectively, at the end of the 30-min recovery period.

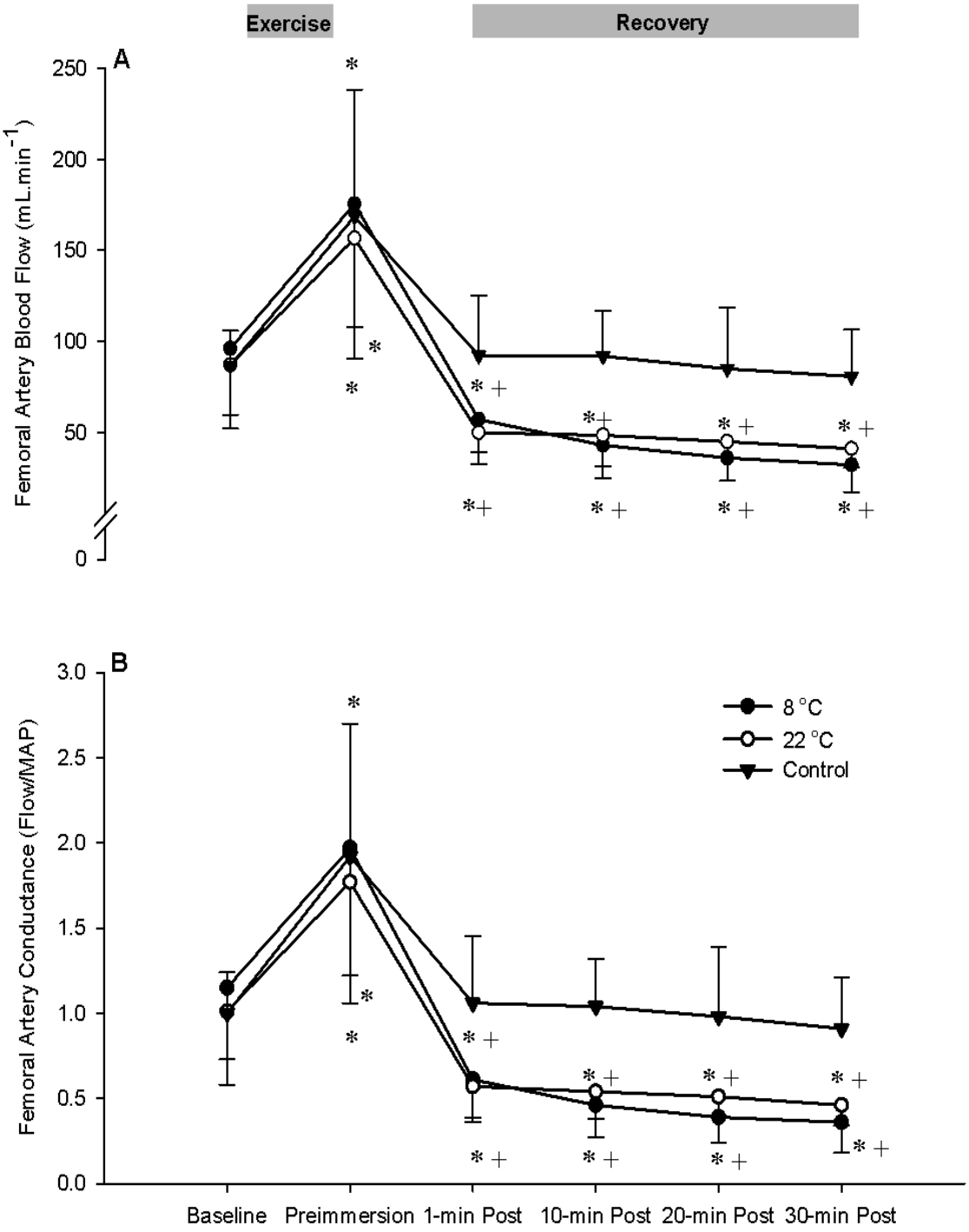

Figure 6.4.5. Femoral artery blood flow (A) and conductance (B) pre and post immersion in 8 °C, 22 °C and CON (n = 12, mean ± SD). A main effect for condition ($P < 0.001$) and time ($P < 0.001$) was found for both artery flow and conductance. There was also a significant interaction between condition and time for both artery flow ($P < 0.01$) and conductance ($P < 0.01$). * Significant difference from baseline ($P < 0.05$). + Significant difference vs CON ($P < 0.05$).

6.4.4. Cutaneous blood flow responses

Pre-immersion thigh (8 °C, 0.23±0.15 AU; 22 °C, 0.28±0.21 AU; CON, 0.31±0.15 AU; $P = 0.31$) and calf (8 °C, 0.22±0.20 AU; 22 °C, 0.16±0.10 AU; CON, 0.17±0.08 AU; $P = 0.45$) CVC were not different between conditions. A greater skin vasoconstriction was observed in both cooling conditions at the thigh ($P < 0.01$) and calf ($P < 0.01$) relative to CON during immersion and throughout the post-immersion recovery period (~50-60 %; $P > 0.05$). No difference was observed between cooling conditions at any time point ($P > 0.05$; Figure 6.4.6).

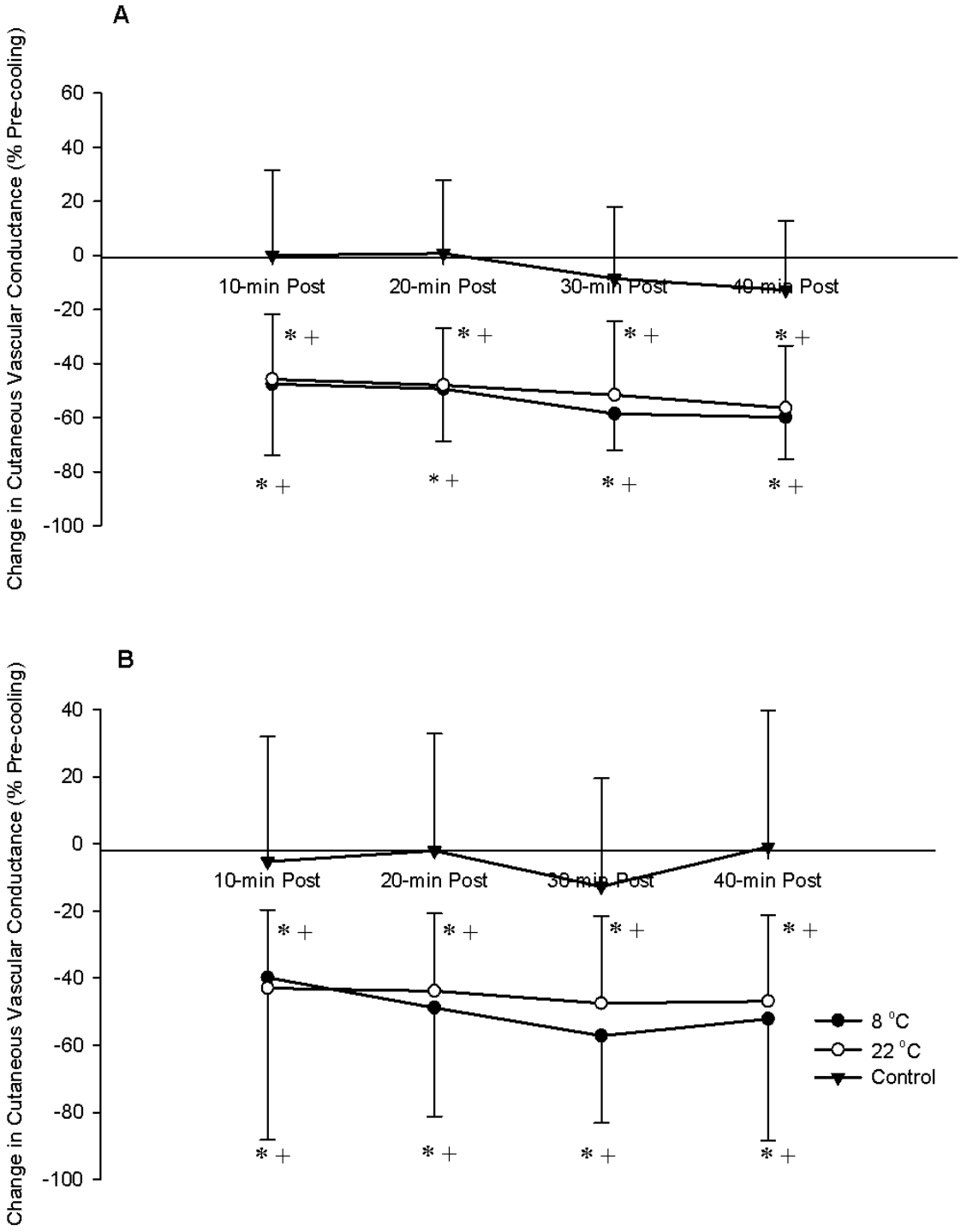

Figure 6.4.6. Percentage change in thigh cutaneous vascular conductance (A) and calf vascular conductance (B) from pre immersion in 8°C, 22°C and CON (n =12, mean ± SD). Main effects for condition (P < 0.01) were found for both thigh and calf cutaneous vascular conductance. A main effect for time (P = 0.01) was also found for thigh conductance. There were no interactions between condition and time in thigh (P = 0.78) or calf vascular conductance (P = 0.42). * Significant difference from Pre immersion (P < 0.05). + Significant difference vs CON (P < 0.05).

6.5. DISCUSSION

The purpose of this study was to investigate the effects of CWI of various water temperatures on lower limb blood flow following resistance exercise. We found no differences in the blood flow responses to CWI at 8 °C and 22 °C following resistance exercise despite greater reductions in T_{muscle} and skin temperatures after CWI of 8 °C. Moreover, these responses were similar in time course and magnitude to our previous findings following endurance cycling exercise (see Chapter 5). Taken together, these findings suggest that the application of CWI is similarly effective with regards to vascular responses following different modes of exercise.

Previous studies, which have examined the influence of CWI on limb blood flow responses after exercise, have used an endurance exercise stimulus (see Chapter 5; Ihsan *et al*., 2013; Vaile *et al.,* 2011). These endurance type protocols typically produce a greater level of systemic (e.g., core temperature) hyperthermia and different metabolic perturbations, compared with resistance exercise (Deschenes *et al*., 1998; Mortensen *et al*., 2008). A relative decrease in blood volume in the leg muscle microcirculation after CWI of 10 °C has been reported after knee extensor resistance exercise using NIRS (Roberts *et al*., 2015a), however, this method is associated with several limitations (Davis *et al*., 2006; Ferrari, Mottola & Quaresima, 2004) compared with absolute measures of femoral and skin blood flow. In the present study, 10-min of lower body immersion in either 8 ºC or 22 ºC water reduced FABF by ~75 % and ~50 %, respectively, compared with the CON condition. The magnitude of change in FVC after CWI was similar to our previous observations (~55 %) after cycling exercise (Chapter 5) and other studies, which assessed limb blood flow with other methods (Ihsan *et al*., 2013; Vaile *et al*., 2011). The lack of difference in the FVC

response to cold (8º C) and cool (22 ºC) water in the current study, despite greater decreases in T_{muscle} in cold water, are in agreement with our previous work [Chapter 5; Gregson *et al*., 2011) and are likely due to an insufficiently large enough difference in deep T_{muscle} between cooling conditions (~1 °C) to directly modify FABF.

In the present study, rises in core (~0.3 ºC) and local limb temperatures (muscle 3 cm, ~1 ºC; skin, ~0.6 ºC) after resistance exercise led to increases in thigh and calf CVC. Despite differences in reductions in lower limb skin temperature after immersion in 8 ºC and 22 ºC water, reductions in lower limb CVC were similar between cooling conditions in agreement with previous work (Chapter 5) that elicited a higher thermoregulatory strain (core 0.9 ºC, muscle 3 cm; 1.6 ºC and skin 1.7 ºC). It is therefore conceivable that only a small hyperthermic load (systemic or local limb) is required to blunt cutaneous vasoconstrictor responsiveness (Wilson, Cui & Crandall, 2002). In addition, cold-induced vasodilation can occur in 8 ºC water, which may contribute to a similar skin blood flow after 8 ºC CWI relative to 22 ºC CWI (Gregson *et al*., 2011). In combination, similar changes in FABF and CBF after CWI in 8 and 22 ºC water suggest that both cooling conditions will be equally effective with regards to the vascular responses when applied after resistance exercise.

It is difficult to directly measure muscle blood flow in humans, particularly across a broad area of muscle. Our approach, measuring total limb and cutaneous blood flows simultaneously, allows some inferences to be drawn regarding generalized changes in blood flow to muscle. In response to cooling in the present experiment, changes in both total limb and cutaneous flows were similar. This suggests that despite distinct impacts of 8 ^{o}C and 22 ^{o}C cooling on skin and muscle temperatures (especially deeper

muscle temperatures), the impact on muscle blood flows was qualitatively similar. Collectively, these data infer that, if different degrees of post-exercise cooling have an impact upon recovery following resistance training, they are independent of blood flow to muscle.

6.6. SUMMARY

The application of lower limb immersion in 8 ºC and 22 ºC water after a bout of resistance exercise decreases FABF and CBF compared with rest and to a similar extent between cold and cool water temperatures. Individuals who may not tolerate colder water temperatures may therefore use less noxious water temperatures after resistance exercise. The present findings also suggest that greater reductions in T_{muscle} with colder water may enhance recovery from soft tissue injury independent of effects on limb blood flow. These findings have practical implications for the use of CWI in clinical and athletic settings to alleviate symptoms of EIMD.

CHAPTER 7

INFLUENCE OF COLD-WATER IMMERSION AND WHOLE BODY CRYOTHERAPY ON LIMB AND CUTANEOUS BLOOD FLOW AFTER EXERCISE

7.1. INTRODUCTION

Whilst the majority of the research literature investigating cryotherapy during recovery from exercise has employed CWI (Ihsan *et al*., 2013; Roberts *et al*., 2015a; Vaile *et al*., 2011), the recent commercial availability of WBC facilities, which expose the body to very cold air (-110 °C to -140 °C) for short durations (2-4 min) (Banfi *et al*., 2010), has led to further interest in the role of cryotherapy in exercise recovery (Bleakley *et al*., 2014; Costello *et al*., 2015). However, the comparative physiological effects of WBC remain to be elucidated.

Previous WBC research studies have shown beneficial impact on haematological profiles (Lombardi *et al*., 2013), inflammatory biomarkers (Pournot *et al*., 2011b, Ziemann *et al*., 2012), muscle damage (Fonda *et al*., 2013; Hausswirth *et al*., 2011, Ziemann *et al*., 2012), oxidative stress (Mila-Kierzenkowska *et al*., 2009; Miller *et al*., 2012), the autonomic nervous system (Hausswirth *et al*., 2013; Schaal *et al*., 2013), body temperature (Costello *et al*., 2012a; Costello *et al*., 2012b; Westerlund *et al*., 2003), functional recovery (Costello, Algar & Donnelly, 2012; Fonda *et al*., 2013) and reductions in tissue oxyhaemoglobin and tissue oxygenation index (Selfe *et al*., 2014). Only one study to date has directly compared the thermoregulatory responses between CWI and WBC recovery interventions. Costello *et al*, (2012a) recently demonstrated that 4 min of exposure to either CWI or WBC similarly decreases core and T_{muscle} for up to 60 minutes post exposure, despite lower Tsk_{thigh} after CWI. However, the CWI duration used in this study was not typical of protocols used for recovery, i.e. ≥10 minutes (Leeder *et al*., 2012; Wilcock, Cronin & Hing, 2006), the cryotherapy modalities were applied under resting conditions and the vascular (blood flow) and hemodynamic responses were not measured. It is therefore currently

unknown which intervention, CWI or WBC, mediates the greatest reduction in blood flow of the previously exercised limb(s). This is important given that reducing blood flow may directly limit muscle damage and function, thus aiding recovery.

7.2. PURPOSE

The aim of the present study was to examine the effects of ecologically valid CWI and WBC protocols on FABF and CBF and thermoregulatory responses after cycling exercise. It was hypothesized that a longer duration of CWI would decrease FABF and lower limb CBF to a greater extent, compared with WBC, and lead to a greater reduction in leg T_{muscle}.

7.3. MATERIALS AND METHODS

7.3.1. Participants

Ten recreationally active men were studied (mean±SD: age, 22.3±3.4 yrs; height, 1.8±0.1 m; mass, 81.1±8.3 kg; BSA, 2.0±0.1 m^2; $\dot{V}O_{2peak,}$ 45±9 mL · kg^{-1} · min^{-1}) and completed all the pretest criteria to participate (see section 3.1.2, 3.1.3 and 3.1.4).

7.3.2. Preliminary testing

Prior to any experimental trials, each participant completed a maximal incremental cycling protocol on a cycle ergometer (Lode, Corival, Netherlands) while simultaneous breath-by-breath ($\dot{V}O_2$) measurements were recorded (Oxycon Pro, Jaeger, Germany). The cycling protocol commenced at 75 W and was increased 25 W every 2 min until volitional exhaustion was reached. Peak power output was derived as the highest power output attained at this point. $\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1}) was recorded as the highest 30 s average recorded prior to volitional exhaustion.

7.3.3 Experimental protocol

Each participant was requested to complete a submaximal cycle ergometer protocol, followed by exposure to either WBC or CWI. The CWI consisted of 10 min of immersion (see section 3.7) The WBC exposure was undertaken in a specialized mobile cryotherapy unit (KrioSystem, Wroclaw, Poland), which lasted for a period of 2 min at a temperature of -110 ºC. Entry to the main chamber was preceded by a 30 *s* adaptation period in a pre chamber at a temperature of -60 ºC. The immersion and WBC protocols were based on methods frequently reported in the literature (Banfi *et al*., 2010, Hausswirth *et al*., 2011; Leeder *et al*., 2012). The conditions were conducted in a counterbalanced order, at least 1 week apart.

On arrival at the laboratory, a rectal probe was self-inserted and a HR monitor was positioned across the chest. Participants were then laid in a supine position for 30 min on a bed for the attachment of instrumentation and to stabilise physiological status (Olive, McCully & Dudley, 2002), wearing training shorts and a tracksuit top. Following baseline measurements, participants cycled at 70 % $\dot{V}O_{2peak}$ until a core temperature of 38 °C was attained. Participants then returned to a supine position for 10 min to enable pre-cooling measurements to be taken. In the CWI condition, participants were subsequently raised from the bed in a semi-recline position using an electronic hoist and lowered into the water bath until the thighs were fully submerged for a duration of 10 min (see section 3.7). In the WBC condition, body sweat was lightly dabbed dry with a towel, and equipment was removed from the body. Skin blood flow and rectal probes remained in situ, with connections covered and tucked inside the participant's shorts and socks. Next, with the help of the researchers, the participants donned the clothing to be worn inside the chamber (face mask, ear band,

gloves, socks and shoes) and were then transferred to and pushed in a chair to undergo WBC exposure inside the chamber. At the end of immersion/WBC protocol, participants were returned to the bed using either the electronic hoist/or via the chair, and remained in a supine position for a period of 40 min under the temperature-controlled laboratory. A period of 10 min was permitted before any post measurements for the reattachment of equipment and doffing of clothing.

T_{rec} (see section 3.2.1), $Tsk_{chest,}$ $TSK_{forearm,}$ $Tsk_{thigh,}$ Tsk_{calf} and Tsk_{mean} (see section 3.2.2), HR (see section 3.5.1) and thigh and calf CBF (see section 3.3) were continuously monitored at baseline, pre-cooling, and during the 40 min post-cooling period. T_{muscle} (see section 3.2.3) was recorded at baseline, immediately pre-cooling and 10 and 40 min post-cooling. FABF (see section 3.4.1) and MAP (see section 3.5.2) were measured at baseline, pre-cooling, and at 10 min intervals during the 40 min post-cooling period. Perceived thermal comfort (see section 3.6.1) and shivering (see section 3.6.2) were also recorded at the same time points. All pre and post-immersion measurements were made in a supine position. A schematic illustration of the experimental design is shown in Figure 7.3.1.

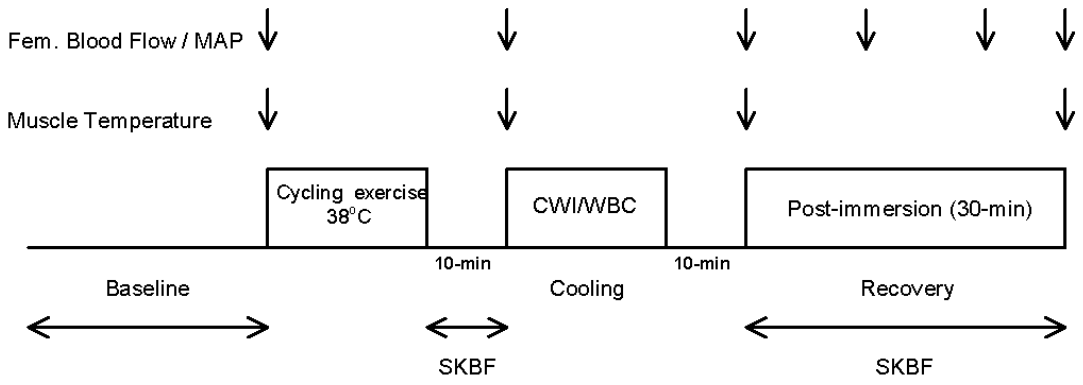

Figure 7.3.1. A schematic of the experimental design

7.3.4. Statistical analysis

It was estimated that a sample size of at least 7 participants would have 90 % power to detect an 83 $mL \cdot min^{-1}$ reduction in FABF following 10 min of cryotherapy (8 °C water immersion), using an SD of the differences of 65 $mL \cdot min^{-1}$ (Study 2) (G*Power, version 3.1, Dusseldorf, Germany). A two-factor (condition x time) GLM was used to evaluate treatment differences between the CWI and WBC conditions. A three-way GLM (condition x depth x time) was used to analyse T_{muscle} (SPSS version 18, Statistical Package for the Social Sciences, Chicago, IL). A significant effect of time was followed up with planned multiple contrasts in line with the *a priori* hypotheses. Consequently, data at the specific time points were compared with the baseline (first) time point using multiple contrasts. As Tsk and thigh and calf CVC was expressed as percentage change from pre immersion (zero), a one-sample t-test was used in follow-up analyses. Therefore, at each measured time-point, the main effect of time for thigh and calf CVC was compared with the pre-immersion (first) time point. A paired-samples t-test was used to determine any differences between conditions at baseline for FVC and at pre immersion for Tsk and thigh and calf CVC. Simple effect size (ES; Hedges' *g*) were estimated from the ratio of the mean difference to the pooled standard deviation (Hedges & Olkin, 1985). The ES magnitude was classified as trivial (<0.2), small (>0.2-0.6), moderate (>0.6-1.2), large (>1.2-2.0) and very large (>2.0-4.0) (Hopkins *et al*., 2009). The statistical significance was set at $P < 0.05$. Data are presented as mean ± SD.

7.4 RESULTS

7.4.1.Thermoregulatory responses

T_{rec} was not different between conditions at baseline (CWI; 37.2±0.2 °C; WBC; 37.2±0.3 °C; P = 0.77, ES = 0.1). The cycling protocol, to attain a core temperature of 38 °C (CWI; 22±4.3 min; WBC; 22.7±7.4 min), elicited a similar increase in T_{rec} (CWI; Δ 0.7±0.3 °C; WBC; Δ 0.7±0.4 °C; P < 0.001; Figure 7.4.1) and was not different between conditions immediately pre immersion (CWI; 37.9±0.2 °C; WBC; 37.9±0.2 °C; P = 0.86, ES = 0.1). T_{rec} decreased over the post cooling recovery period (P < 0.001) and was similar between conditions (P = 0.98, ES = 0.1; Figure 7.4.1). T_{rec} returned to baseline values at the end of the post-cooling recovery period in both conditions (P > 0.05).

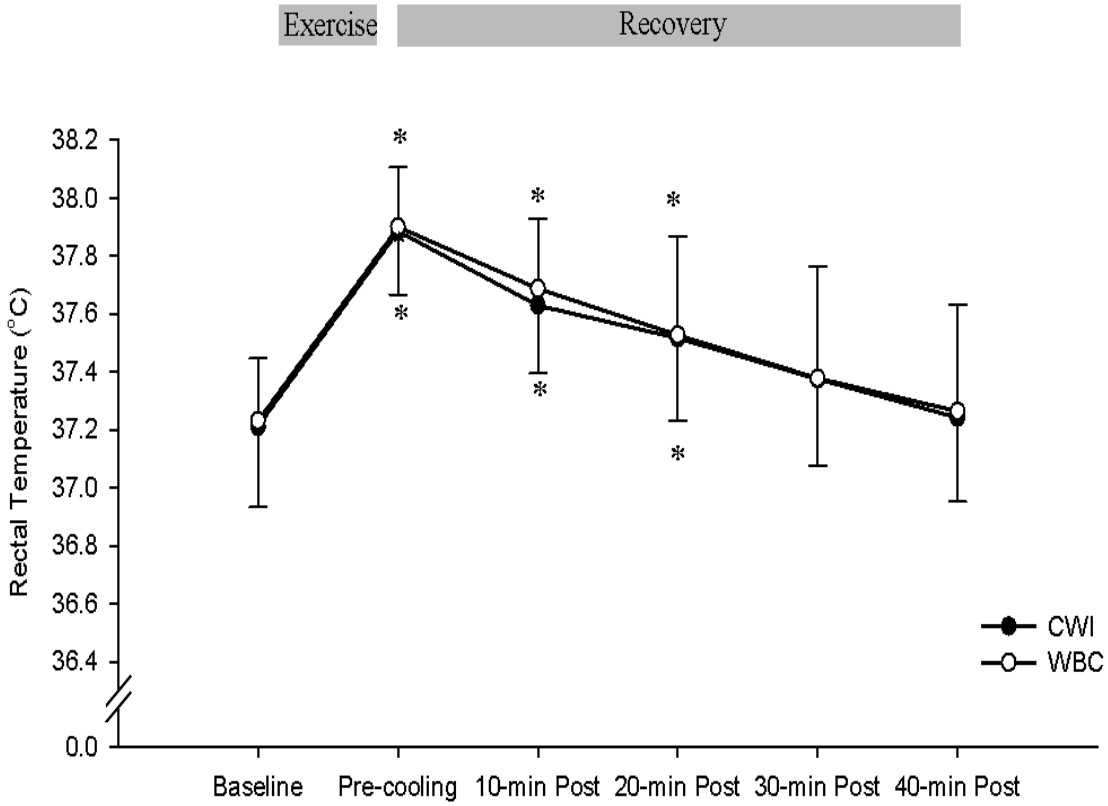

Figure 7.4.1. Rectal temperature pre and post cooling in CWI and WBC (n = 10, mean ± SD). A main effect for time ($P < 0.001$) was found for rectal temperature. * Significant difference from baseline in each condition ($P < 0.001$).

At baseline, both Tsk_{thigh} (CWI; 32.2±1.5 °C; WBC; 30.3±1.0 °C; $P = 0.02$, ES = 1.4) and Tsk_{mean} (CWI; 32.8±0.9 °C; WBC; 30.2±0.7 °C; $P < 0.01$, ES = 3.0) were different between conditions. Tsk_{mean} generally increased ($P < 0.001$) after exercise (i.e. immediately pre cooling) (CWI; Δ 0.3±1.2 °C; WBC; Δ 1.1±0.9 °C), however Tsk_{thigh} generally remained similar ($P = 0.34$) to baseline values (CWI; Δ 0.9±1.1 °C; WBC; Δ 1.3±1.0 °C). There was a difference between conditions immediately pre cooling in Tsk_{thigh} (CWI; 33.1±1.0 °C; WBC; 31.5±1.0 °C; $P = 0.02$, ES = 1.5) and Tsk_{mean} (CWI; 33.09±1.6 °C; WBC; 31.4±0.8 °C; $P = 0.02$, ES = 1.3). A greater rate of decrease in both Tsk_{thigh} ($P < 0.001$) and Tsk_{mean} ($P < 0.001$) occurred in the CWI condition with the largest difference occurring 10 min post-cooling (Tsk_{thigh}; CWI, Δ -9.1±1.9 °C; WBC, Δ -1.6±1.4 °C; $P < 0.001$, ES = 4.3; Tsk_{mean}; CWI, Δ -4.1±0.9 °C;

WBC, Δ -3.2±1.1 °C; $P < 0.001$, ES = 0.8). Tsk_{thigh} gradually increased during the 10-40 min recovery period in the CWI condition whilst values remained relatively stable in WBC. Tsk_{mean} gradually increased in both cooling conditions over the same period. There was a difference in Tsk_{thigh} between CWI and WBC conditions at each post cooling time point ($P < 0.001$). A difference between conditions was also noted in Tsk_{mean} at 10 min ($P < 0.01$) and 40 min ($P < 0.01$) post cooling time points. At the end of the recovery period, Tsk_{thigh} remained below baseline in the CWI condition (27.4±1.3 °C; Δ 4.9±1.8 °C; $P < 0.001$) and unchanged in WBC (30.5±1.2 °C; Δ 0.2±0.5 °C; $P = 0.96$; Figure 7.4.2).

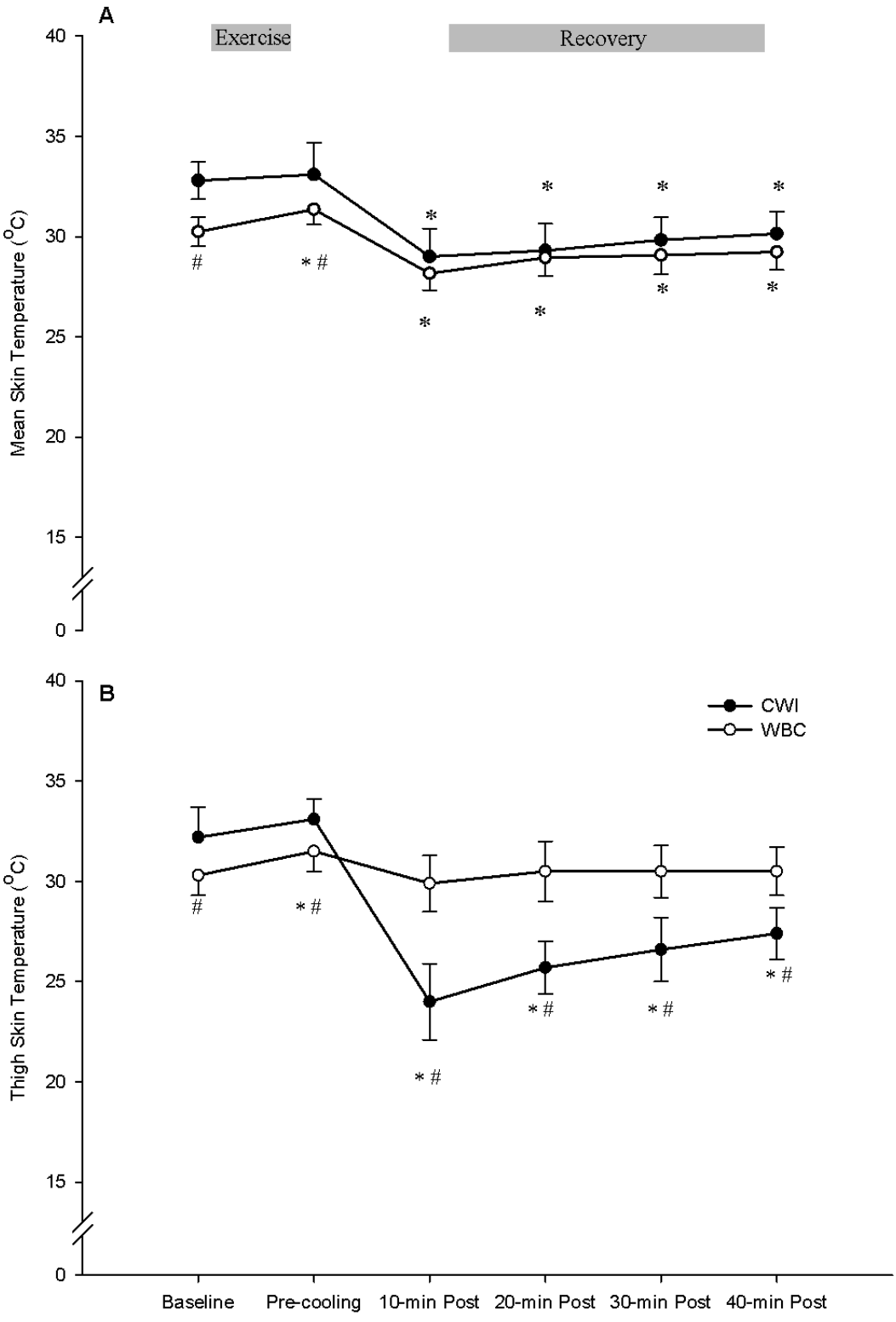

Figure 7.4.2. Percentage change in mean skin temperature (A) (n = 7) and thigh skin temperature (B) (n =10) from pre-cooling in CWI and WBC (mean ± SD). Main effects for condition ($P < 0.001$) were found for Tsk_{mean} ($P = 0.02$) and Tsk_{thigh} ($P < 0.001$). Main effects for time were found for both Tsk_{mean} ($P < 0.01$) and TSK_{thigh} ($P < 0.001$) alongside significant interactions between condition and time in Tsk_{mean} ($P = 0.02$) and Tsk_{thigh} ($P < 0.001$). * Significant difference from pre-cooling ($P < 0.05$). # Significant difference between cooling conditions $P < 0.05$).

Baseline T_{muscle} was similar between conditions at 3 cm (CWI; 36.1±0.7 °C; WBC; 36.0±0.4 °C, P = 0.7, ES = 0.2), 2 cm (CWI; 35.5±0.8 °C; WBC; 35.2±0.7 °C, P = 0.2, ES = 0.4) depths but was different at a depth of 1 cm (CWI; 34.8±0.9 °C; WBC; 34.1±0.9 °C, P = < 0.01, ES = 0.7). The cycling protocol increased T_{muscle} from baseline at 3 cm (CWI; 37.6±0.2 °C; WBC; Δ 37.6±0.3 °C; P < 0.001), 2 cm (CWI; 37.2±0.3 °C; WBC; 37.1±0.3 °C; P < 0.001), and 1 cm (CWI; 36.4±0.9 °C; WBC; 36.1±0.9 °C; P < 0.001) probe depths and was similar between conditions at each depth immediately pre-cooling (P > 0.05; ES = 0-0.3; Figure 7.4.3). T_{muscle} was reduced 10 min following cooling in both conditions at 3 cm (CWI, 35.6±0.5 °C; WBC, 36.7±0.2 °C; P < 0.001), 2 cm; (CWI, 33.1±1.4 °C; WBC, 35.8±0.5 °C; P < 0.001) and 1 cm (CWI, 30.9±1.5 °C; WBC, 34.5±0.7 °C; P < 0.001) depths. T_{muscle} was also reduced in both cooling conditions at 40 min post cooling at 3 cm (CWI, 34.7±0.9 °C; WBC, 36.3±0.5 °C; P < 0.001); 2 cm (CWI, 33.3±1.3 °C; WBC, 35.5±0.8 °C; P < 0.001) and 1 cm (CWI, 32.0±1.4 °C; WBC, 34.2±1.0 °C; P < 0.001) depths. The reduction in T_{muscle} at each depth was greater after CWI compared with WBC at both 10 min (1 cm; P < 0.001, ES= 1.7; 2 cm; P < 0.001, ES = 1.9; 3 cm; P < 0.001, ES = 2.8) and 40 min (1 cm; P < 0.001, ES = 1.4; 2 cm; CWI, P < 0.001, ES = 1.4; 3 cm; P < 0.001, ES = 2.1) time points (Figure 7.4.3).

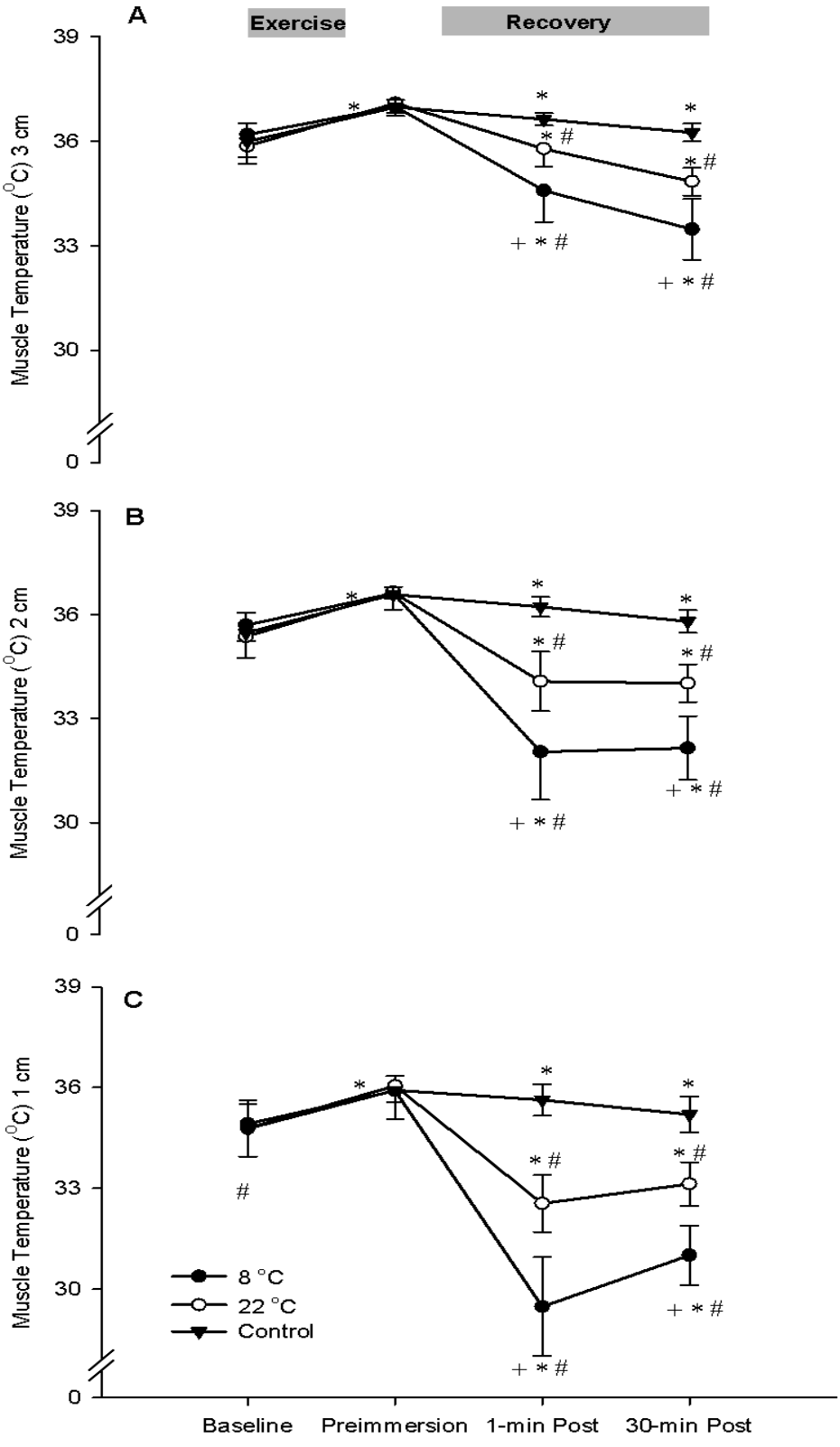

Figure 7.4.3. Muscle temperature pre and post cooling at temperature probe depths of 3 cm (A), 2 cm (B), and 1 cm (C) (n =10, mean ± SD). Main effects for condition ($P < 0.001$) and time ($P < 0.001$) were found along with a significant interaction among condition, time and probe depth ($P < 0.001$). * Significant difference from baseline ($P < 0.001$). # Significant difference between cooling conditions ($P < 0.001$).

Thermal comfort was similar between conditions at baseline (CWI, 5±1 AU; WBC, 4±1 AU; $P = 0.07$, ES = 1.0) and immediately pre immersion (CWI, 7±1 AU; WBC, 6±2 AU; $P = 0.07$, ES = 0.6). A greater rate of decrease in thermal comfort ($P < 0.001$) was reported after CWI at 10 min (CWI, 4±1 AU; WBC, 5±1 AU; $P = 0.02$, ES = 1.0) and 20 min post cooling (CWI, 4±1 AU; WBC, 5 ± 1 AU; $P = 0.04$, ES = 1.0) compared with WBC. Thermal comfort was similar between conditions at 30 min post cooling (CWI, 4±1 AU; WBC, 5±1 AU; $P = 0.2$, ES =1.0) and until the end of the 40 min recovery period (CWI, 5±1 AU; WBC, 5±1 AU; $P = 1.0$, ES = 0.1). There was no shivering observed throughout the post immersion period under any experimental condition.

7.4.2 Heart rate and mean arterial pressure

HR was similar between cooling conditions at baseline (CWI; 65±9 beats·min^{-1}; WBC; 63±8 beats·min^{-1}; $P = 0.96$, ES = 0.2). Average HR was not different during the cycling protocol (CWI; 156±10 beats·min^{-1}; WBC; 156±7 beats·min^{-1}; $P = 0.96$, ES = 0.1) and was similar between conditions immediately pre-cooling (CWI; 92±11 beats·min^{-1}; WBC; 94±11 beats·min^{-1}; $P = 0.27$, ES = 0.2; Figure 7.4.4). HR decreased throughout the recovery period in both conditions ($P < 0.001$). A lower HR was observed in CWI at 10 min (CWI, 72±12 beats·min^{-1}; WBC, 82±13 beats·min^{-1}; $P < 0.001$, ES = 0.8), and 20 min post cooling (CWI, 71±9 beats·min^{-1}; WBC, 78±12 beats·min^{-1}; $P < 0.01$, ES = 0.6) but then remained similar to WBC until the end of the recovery period (CWI, 67±9 beats·min^{-1}; WBC, 73±10 beats·min^{-1}; $P > 0.05$, ES = 0.6). HR was unchanged from baseline values in CWI from 20 min post cooling until the end of the recovery period (67±9 beats·min^{-1}; $P > 0.05$) but remained above baseline in WBC (73±10 beats·min^{-1}; $P < 0.001$; Figure 7.4.4).

MAP was similar between conditions at baseline (CWI; 85±5 mmHg; WBC; 87±7 mmHg; P = 0.25, ES = 0.3), with values unchanged (P > 0.05) immediately precooling (CWI; 87±8 mmHg; WBC; 85±7 mmHg; P = 0.20, ES = 0.2) (Figure 7.4.4). However, MAP was higher 10 min post cooling in the CWI condition compared with WBC (CWI; 90±6 mmHg; WBC; 86±8 mmHg; P = 0.04, ES = 0.5). Similar values were subsequently were noted until 40 min post immersion where higher MAP values were recorded in the CWI condition (CWI; 93±8 mmHg; WBC; 85±9 mmHg; P < 0.001, ES = 0.9). MAP remained above baseline values in CWI (P = 0.001) but was unchanged in WBC (P = 0.85) at the end of the recovery period (Figure 7.4.4).

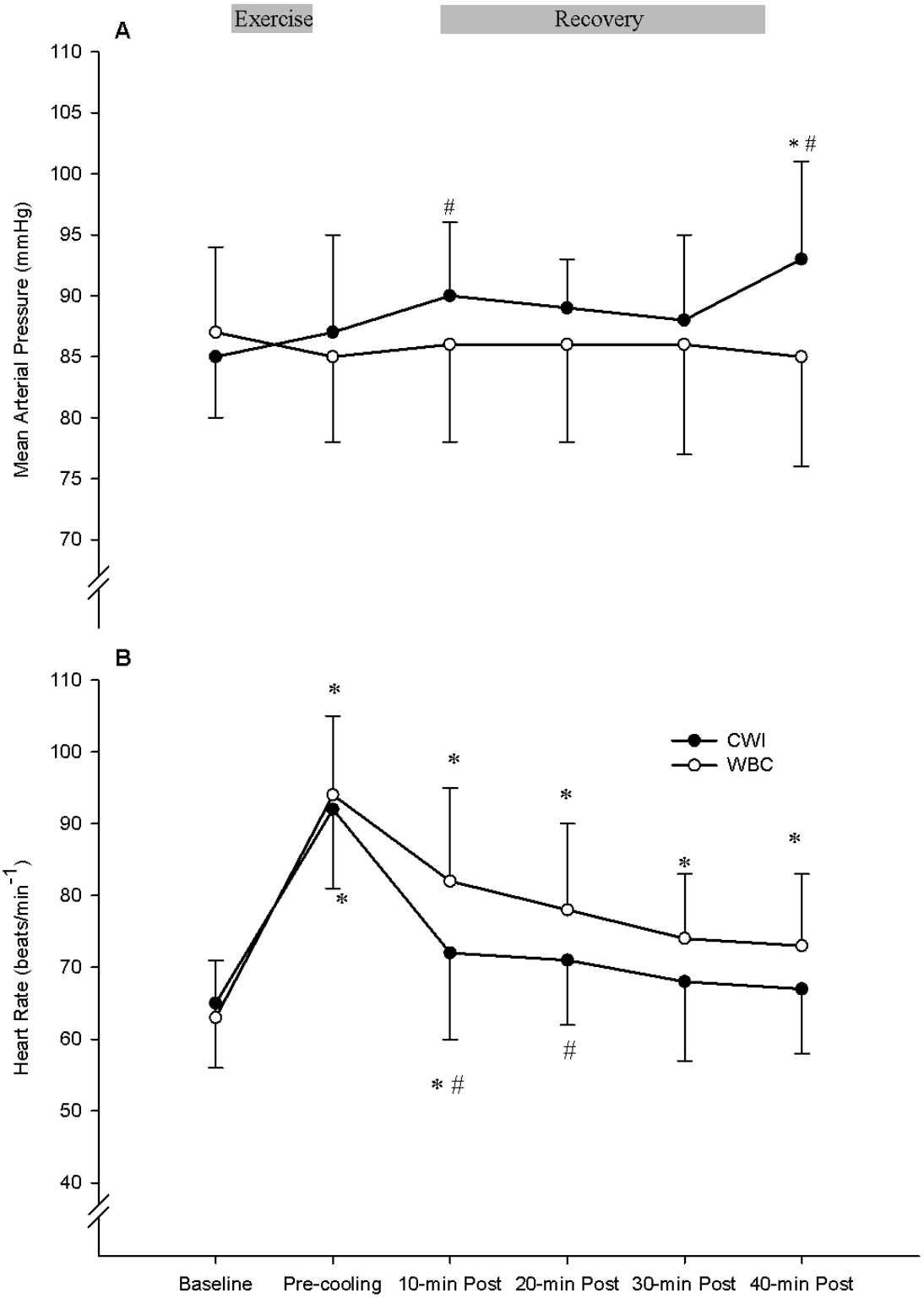

Figure 7.4.4. Mean arterial pressure (n = 10), (A) and heart rate (n = 12), (B) pre and post cooling in CWI and WBC (mean ± SD). Main effects for condition ($P = 0.01$) and interaction ($P = 0.02$) were found for MAP. A significant interaction effect was also found for HR ($P = 0.001$) alongside a main effect for time ($P = 0.001$). * Significant difference from baseline ($P < 0.05$). # Significant difference between cooling conditions ($P < 0.05$).

7.4.3 Femoral artery blood flow responses.

FABF and FVC were similar between conditions at baseline and post-exercise (pre-cooling) ($P > 0.05$). Exercise increased FABF and FVC by ~65-70 % ($P < 0.001$) (Figure 7.4.5). FABF and FVC were reduced 10 min post cooling by ~50 % and ~70 % in the WBC and CWI conditions, respectively ($P < 0.001$). The rate of decrease in FABF was different between conditions ($P < 0.001$) with moderate differences occurring at 10 min ($P = 0.06$, ES = 0.8), 20 min ($P = 0.04$, ES = 0.9), 30 min ($P = 0.03$, ES = 1.0) and 40 min ($P = 0.02$, ES = 1.1) post cooling. The rate of decrease in FVC was also different between conditions ($P < 0.001$), with moderate differences occurring throughout the recovery period (10 min; $P = 0.04$, ES = 1.0; 20 min; $P = 0.03$, ES = 1.0; 30 min; $P = 0.02$, ES = 1.1; 40 min; $P = 0.01$, ES = 1.2). At 40 min post recovery, FABF and FVC were (~45-50 %) lower in CWI compared with WBC. Relative to baseline, FABF and FVC were reduced by ~35 % at the end of the recovery period in the CWI condition ($P > 0.05$). In comparison, during WBC FABF and FVC remained ~30 % above baseline values ($P > 0.05$; Figure 7.4.5).

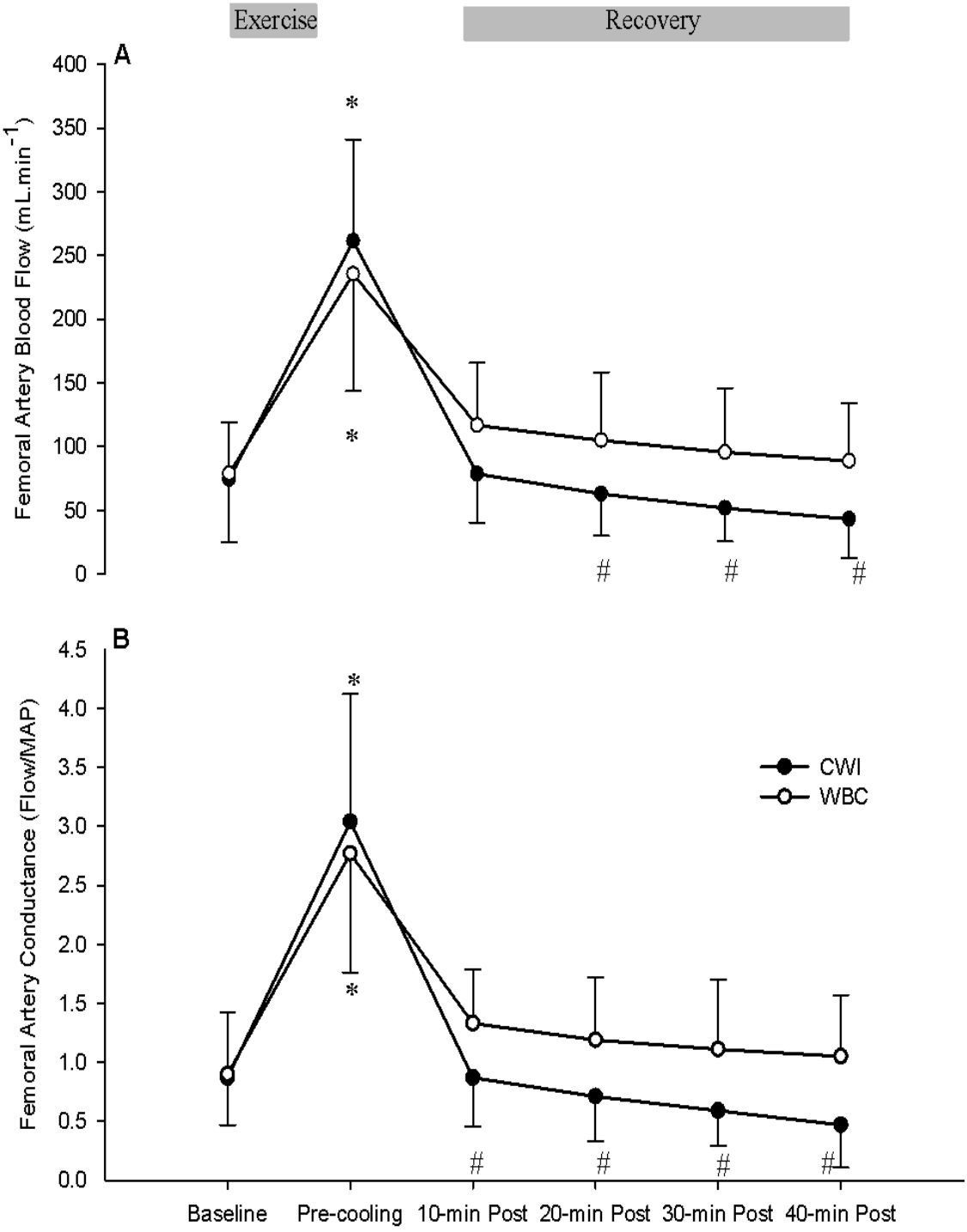

Figure 7.4.5. Femoral artery blood flow (A) and conductance (B) pre and post cooling in CWI and WBC (n = 10, mean ± SD). A main effect for time ($P < 0.001$) was found for both artery flow and conductance. There was also a significant interaction between condition and time for both artery flow ($P = 0.004$) and conductance ($P = 0.002$). * Significant difference from baseline ($P < 0.001$). # Significant difference between cooling conditions ($P < 0.05$).

7.4.4 Cutaneous blood flow responses.

CVC was similar between conditions immediately pre-cooling in the thigh (CWI, 0.4±0.2 AU; WBC, 0.4±0.3 AU; P = 0.93, ES = 0.1) but was different at the calf (CWI, 0.4±0.2 AU; WBC, 0.2±0.1 AU; P < 0.01, ES = 1.2). A greater skin vasoconstriction relative to pre cooling was observed in the CWI condition compared with WBC at the thigh (~75 % *vs.* ~55 %; P < 0.001, ES = 1.9) and calf (~70 % *vs.* ~45 %; P < 0.001, ES = 1.6) throughout the recovery period (Figure 7.4.6).

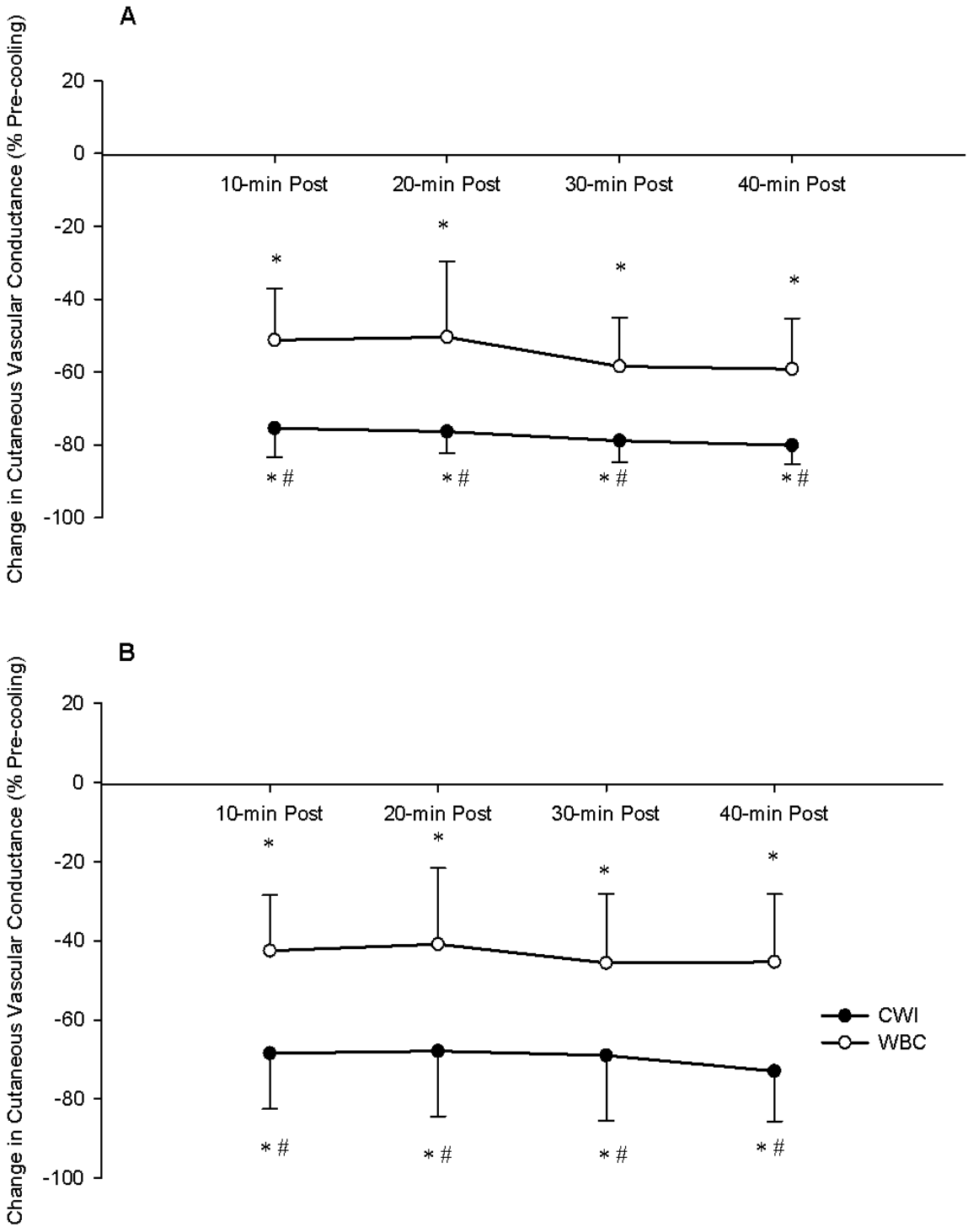

Figure 7.4.6. Percentage change in thigh cutaneous vascular conductance (A) and calf vascular conductance (B) from pre cooling in CWI and WBC (n =10, mean ± SD). Main effects for condition were found for both thigh ($P = 0.002$) and calf ($P < 0.001$) cutaneous vascular conductance. A main effect for time ($P = 0.001$) was also found for thigh conductance. There were no interactions between condition and time in thigh ($P = 0.44$) or calf vascular conductance ($P = 0.52$). * Significant difference from pre cooling ($P < 0.001$). # Significant difference between cooling conditions ($P < 0.01$).

7.5. DISCUSSION

The major finding of the present study is that, relative to WBC, CWI led to greater reductions in FABF and CBF, as well as deep and superficial T_{muscle}, during the post-exercise recovery period. Collectively, our novel data provide evidence that CWI after cycling exercise may potentially reduce muscle blood flow to a greater extent than WBC. These findings provide important insights into the relative efficacy of, and the possible mechanisms that underpin, distinct cryotherapy recovery modalities commonly used in clinical and sporting environments.

To date, only one study has previously attempted to document the limb blood flow response to WBC cooling using the NIRS technique (Selfe *et al*., 2014). On the morning after exercise (rugby league match) significant reductions in tissue oxyhaemoglobin and deoxyhaemoglobin and tissue oxygenation index were evident immediately after 3 min of WBC, which caused a reduction in abdominal skin temperature of ~15 °C. The NIRS method provides indirect estimates of relative changes in blood volume within the muscle microcirculation but is associated with a number of limitations related to the NIRS signal (Ferrari, Mottola & Quaresima, 2004). Importantly, NIRS measures of tissue oxygenation may be confounded when marked changes in skin blood flow arise (e.g. exercise, heating, cooling; Davis *et al*., 2006). In line with study 2 and previous observations under resting conditions (Gregson *et al*., 2011), the present investigation measured changes in lower limb CBF using laser Doppler flowmetry while simultaneously measuring FABF via conduit artery high-resolution duplex ultrasound after WBC. By simultaneously measuring the effects of cooling on FABF and CBF within the limb, qualitative effects of cooling on muscle perfusion may be inferred (Study 2; Gregson *et al*., 2011).

Presently, only one study (Costello *et al*., 2012a) has compared the thermoregulatory responses (i.e., T_{rec}, T_{muscle} and Tsk) between CWI and WBC recovery modalities. However, the duration of CWI (4 min) was not representative of a CWI protocol typically used for recovery in various sporting environments, i.e. ≥ 10 min (Leeder *et al.,* 2012, Wilcock, Cronin & Hing, 2006) and neither modality was applied after exercise. Therefore, the vascular and thermoregulatory responses to CWI compared to WBC after exercise are unknown and it is unclear which cryotherapy modality might provide the greatest benefit for recovery after exercise, based on changes in these key physiological indices.

The different physiological reactions to WBC and CWI are likely dependent on the magnitude of decreases in core and local tissue temperatures. In agreement with previous findings (Costello *et al*., 2012a), no difference was observed in recovery T_{rec} between cooling modalities. In line with Costello *et al's* (2012a) results, we also noted a lower Tsk after CWI throughout the recovery period. These findings are likely to be related to the greater conductance of tissue heat transfer/loss in water compared with air (Westerlund *et al*., 2003) and/or the greater duration of CWI cooling used in the current study.

In the present investigation, FVC was reduced 40 minutes after cooling by ~60 % and ~85 % relative to pre immersion values in the WBC and CWI conditions, respectively. The magnitude of reduction in FVC after CWI is comparable to Study 2. Whilst it is difficult to directly compare studies that use different limb blood flow measurement techniques, our findings also confirm NIRS observations of reductions in indexes of muscle blood volume after WBC (Selfe *et al*., 2014). To our knowledge,

this is the first study, which has compared the limb blood flow responses to CWI and WBC cooling modalities. There was a ~50 % difference in the reduction of FVC between cooling modalities at the end of the recovery period. In practical terms, the difference of FABF of ~50 $mL \cdot min^{-1}$ (moderate magnitude of ES; ~1-1.2) between conditions is of strong physiological relevance particularly when this difference is evident over at least 40 min (Figure 7.4.5) and has clear implications for the edema and the inflammatory response to exercise (Lee *et al*., 2005; Thorlacius *et al*., 1998). The reduction in arterial blood flow is mediated via activation of thermonociceptors during skin cooling, which leads to a reflex increase in sympathetic nerve activity (Gregson *et al*., 2011, Lovallo, 1975). The differences in arterial blood flow between CWI and WBC may therefore be related to the different thermal input associated with skin cooling during and post cooling in both recovery modalities.

Alongside the changes in FABF, CBF was reduced throughout the recovery period relative to pre immersion in both CWI (~70-75 %) and WBC (~45-55 %) conditions, with a greater vasoconstriction observed after CWI. There is little available literature to compare the cutaneous responses to WBC and CWI, possibly due to methodological issues, however the cutaneous vasoconstriction after CWI is consistent with previous observations after rest (Gregson *et al*., 2011) and exercise (Study 2). The moderate magnitude of difference in total limb flow (i.e. femoral artery; see above), and large difference in cutaneous flow (ES = ~1.6–1.9) throughout the recovery period in CWI compared with WBC suggests that CWI potentially reduces muscle blood flow to a greater extent. Taken together, these data infer that CWI may have a greater impact upon reducing edema and the inflammatory response

(Lee *et al*., 2005, Thorlacius *et al*., 1998) to benefit recovery following endurance exercise.

To date, only one study (Costello *et al*., 2012a) has compared the thermoregulatory responses (i.e., core, T_{muscle} and Tsk) between CWI and WBC recovery modalities. However, the duration of CWI (4 min) was not representative of the CWI protocol typically used for recovery in various sporting environments, i.e. ≥ 10 min (Leeder *et al*., 2012, Wilcock, Cronin & Hing, 2006) and neither modality was applied after exercise. Therefore, in addition to vascular changes, the thermoregulatory responses to CWI compared to WBC after exercise has yet to be fully elucidated.

Muscle temperature-induced reductions in microvascular blood flow may reduce edema and the inflammatory response after tissue injury (Lee *et al*., 2005; Thorlacius *et al*., 1998). Our findings suggest that deep and superficial T_{muscle} were reduced after both CWI and WBC cooling, with lower T_{muscle} observed after CWI. In line with previous findings, where CWI has been applied under resting (Gregson *et al*., 2011) and after exercise (Study 2) conditions, deep (3 cm) T_{muscle} decreased below baseline after 10 minutes and continued to decrease over the 40 min recovery period. Superficial (1 cm) T_{muscle} also initially decreased below baseline 10 minutes after immersion before returning towards baseline (Figure 7.4.3). As previously described (Enwemeka *et al*., 2002, Gregson *et al*., 2011), our data represents conductive heat loss from the deeper warmer tissues to superficial tissue upon removal of the cooling source, leading to a gradual increase in superficial temperature and a decrease in deep T_{muscle} over time. In the WBC condition, T_{muscle} gradually decreased at both superficial and deeper tissue depths after cooling but remained above baseline throughout the recovery period. This suggests WBC has minimal impact on T_{muscle}

during the post-cooling period and may be less effective than CWI in mediating limb blood flow, edema and the inflammatory response after tissue injury. These observations are in line with an expected greater conductance of tissue heat transfer/loss in water compared with air (Westerlund *et al*., 2003). However, our findings contrast with previous observations, which reported similar reductions in thigh T_{muscle} between CWI and WBC modalities (Costello *et al*., 2012a). The disparity with our findings is likely to be related to Costello *et al* (2012a) using a matched CWI and WBC exposure duration (4 min), and/or the application of cryotherapy under resting conditions.

The greater post-cooling T_{muscle} variation in CWI compared with WBC (Figure 7.4.3) may be related to individual characteristics, which can impact on heat conductivity. For example, body mass, body fat levels, body surface area, body surface area to mass ratio and perfused skin and muscle (Stephens *et al*., 2016), in combination with the different heat conductivity between air and water, may have caused a greater T_{muscle} variance in CWI.

The difference in FVC between cooling modalities may have reflected greater changes in deep T_{muscle} during CWI modulating the limb blood flow response (Barcroft & Edholm, 1943). The pattern of change in FVC mirrored that of deep T_{muscle} in that the differences between CWI and WBC became larger as the post-cooling recovery period progressed. Previous work work (Study 2, Chapter 5) showed that a relatively small change in deep T_{muscle} (0.5 °C) between different degrees of immersion cooling did not influence FVC. In the present study, limb blood flow was significantly lower during CWI recovery, alongside a significantly lower deep T_{muscle} (10 min 1.1 °C and 40 min 1.6 °C) during CWI recovery. These data suggest that

differences in deep T_{muscle} of >1.0 °C likely modulated limb blood flow.

7.6. SUMMARY

This study demonstrates that an ecologically valid CWI protocol decreases both FABF and CBF and T_{muscle} to a greater extent compared with a typical WBC protocol after endurance exercise. Therefore, CWI may be a better recovery modality to enhance recovery from soft tissue injury due to greater reductions in T_{muscle} and possible alterations in muscle blood flow. These findings have practical implications for the use of cryotherapy in athletic settings to alleviate the symptoms of EIMD.

CHAPTER 8

SYNTHESIS OF FINDINGS

The aim of this chapter is to interpret and integrate the findings obtained within this thesis. The initial section provides an overview of the findings in relation to the original aims and objectives before discussing in more detail in the general discussion. The findings are related to the advancement of current knowledge of vascular and thermoregulatory mechanisms associated with the application of CWI after exercise. Finally, the limitations and recommendations for future research are outlined and a general conclusion is presented.

8.1. REALISATION OF AIMS AND OBJECTIVES

The reliability of duplex Doppler ultrasound in the measurement of FABF was initially studied (Aim 1) to permit future investigations into the influence of CWI on limb blood flow after exercise and to provide an estimate of the required sample sizes. It was established that duplex Doppler ultrasound could be reliably used (with minimal systematic bias) both within and between-days to collect measurements of FABF and FVC under resting conditions. Utilizing the Doppler ultrasound technique, the influence of different degrees of water immersion on limb and CBF and thermoregulatory responses after endurance exercise were then examined (Aim 2). It was established that Cold (8 °C) and Cool (22 °C) water immersion decreased both FABF and CBF to a similar extent compared with CON after cycling exercise. T_{muscle} was observed to be lower in the 8 °C water condition, indicating colder water temperatures may further enhance recovery post endurance exercise due to greater decreases in T_{muscle} *per se* and not muscle blood flow. This study was progressed to determine the effects of similar water temperatures on limb and CBF and thermoregulatory responses after resistance exercise; which elicits a different thermal and haemodynamic status on the body (Aim 3). It was found that Cold (8 °C) and

Cool (22 °C) water immersion decreased both FABF and CBF to a similar extent compared with CON after resistance (squat) exercise. In line with the findings after endurance exercise (Chapter5) it was observed that T_{muscle} was lower in the 8 °C water condition. This suggests any further beneficial effects of colder water temperatures on recovery post resistance exercise may be due to greater decreases in T_{muscle} *per se* and not muscle blood flow. In the final study of this thesis, the influence of CWI was compared against another popular mode of cryotherapy, WBC, on limb and cutaneous blood flow and thermoregulatory responses after endurance exercise (Aim 4). It was found that CWI (8 °C) decreased FABF and CBF to a greater extent compared with WBC (-110 °C). Furthermore, it was observed that CWI caused greater decreases in T_{muscle} compared with WBC. These findings suggested that CWI may be a better recovery strategy option to alleviate symptoms of EIMD compared with WBC due to potential greater reductions in both muscle blood flow and T_{muscle}.

8.2. GENERAL DISCUSSION

Recovery strategies are frequently used after athletic training or competition to speed up the recovery process and assist in preventing decrements in physiological and muscular function. Whilst CWI is a widely used post-exercise recovery method, the physiological mechanisms responsible for its use are not well understood. Since inflammation is integral in the development of EIMD, it is thought that a reduction in inflammation, via temperature-dependent reductions in microvascular blood flow, is beneficial to recovery after CWI (Lee *et al*., 2005; Thorlacius *et al*., 1998). However, the influence of different degrees of water immersion on muscle perfusion after exercise has not been elucidated.

The Doppler ultrasound method allows for continuous absolute blood flow measures in a conduit artery, e.g. femoral artery; that is deemed to primarily perfuse muscle and skin (Dinenno *et al*., 1999). Utilizing this technique alongside simultaneous measures of skin blood flow permits estimates of changes in muscle blood flow (Gregson *et al*., 2011). This method has advantages over VOP and NIRS techniques, which have previously been used to assess changes in whole limb blood flow (Vaile *et al*., 2011) and changes in muscle blood volume (Ihsan *et al*., 2013; Roberts *et al*., 2015a), respectively, after CWI has been applied after exercise. The first study in Chapter 4 showed that Doppler ultrasound insonation provides reliable measurements of FABF under resting conditions. This was important to establish since FABF was a key measure in the assessment of changes in muscle perfusion in Chapters 5, 6 and 7 and permitted sample size estimations. The relatively small CV (16-20 %) calculated for within and between-day MBF measurements confirmed previous observations (~10-18 %) reported in the femoral artery (Dinenno *et al.,* 1999; de Goot *et al.,* 2004) and brachial artery (Green *et al*., 2002) using the Doppler ultrasound method. Similarly, these findings were consistent with forearm and calf VOP blood flow measurements (CV, ~11-13 %) (Roberts, Tsao & Breckenridge, 1986). It was established that the measurement error related to Doppler ultrasound assessment of FABF predominantly originated from MBV. This is not surprising since there is intrinsic heart rate variability associated with changes in blood velocity (Eriksen *et al.,* 1990) and MBV is used in the calculation of MBF. Therefore, in Chapter 4, it was determined that duplex Doppler ultrasound could be used to collect repeated measurements of FABF alongside laser Doppler CBF measures to provide an estimate of changes in muscle blood flow in future chapters.

In Chapter 5, it was established that cold and cool water immersion reduces FABF (~50 %) and CBF to a similar degree compared with a CON (non-immersion) after cycling exercise. These findings have implications to recovery since CWI is frequently applied immediately after endurance exercise when body temperature and limb blood flow are significantly elevated. The present results contrast with observations under resting conditions (Gregson *et al*., 2011), which used a similar compartmental blood flow assessment, water temperatures and duration of immersion. Whilst Gregson *et al* (2011) reported FABF to also decrease to a similar extent (~ 40 %) after 8 °C and 22 °C water immersion, a greater CBF was observed in the colder water. The authors attributed this difference in CBF to the occurrence of cold-induced vasodilation in the skin at colder water temperatures. These findings have implications to recovery since a higher skin blood flow may redistribute blood from the underlying muscle, suggesting colder water could be considered more effective in reducing muscle blood flow and inflammation at rest. In Chapter 5, these findings were not replicated since the associated increases in core and limb temperatures (core, 0.9 ºC, T_{muscle} 3 cm, 1.6 ºC; Tsk_{thigh}, 1.7 ºC) may have led to a sympatholytic effect in the skin (Wilson, Cui & Crandall, 2002). This may have prevented skin blood flow reaching low levels and the associated onset of cold-induced vasodilation, leading to sustained vasoconstriction in both 8 ºC and 22 ºC water. This may provide an explanation for the similar CBF noted between cooling conditions.

The magnitude of reduction in FABF using cold and cool water temperatures is consistent with VOP (Vaile *et al*., 2011) and NIRS (Ihsan *et al*., 2013; Roberts *et al*., 2015a) changes in whole limb blood flow and blood volume, respectively, after post-exercise CWI. Interestingly, a similar reduction in FABF was observed between

cooling conditions despite greater decreases in T_{muscle} in the colder water. Whilst an elevated body temperature can impair vasoconstrictor responsiveness in the skin, this does not occur in the whole limb (Keller *et al*., 2010). This suggests that the ~0.5 °C difference noted in deep T_{muscle} between cooling conditions was not of a sufficient magnitude to modify FABF. Despite these findings, pragmatists would argue that greater clinical benefits still result from greater reductions in T_{muscle} (Bleakley & Hopkins, 2010). Indeed, a primary rationale of cold application is to reduce the metabolic rate of otherwise uninjured tissue to survive secondary ischemic injury (Merrick, Jutte & Smith, 2003) and progression of EIMD (White & Wells, 2013). Therefore, the greater reductions in T_{muscle}, which arose from the colder water, may be more efficacious in the treatment of EIMD and injury after endurance exercise. Nevertheless, based on key physiological changes (limb blood flow and T_{muscle}), cool water temperatures still appear to benefit recovery compared with rest and may be offered as an alternative to athletes who cannot tolerate colder water temperatures.

In Chapter 6, the thesis was progressed by using resistance exercise to examine the effects of a lower core and limb temperature (skin and muscle) on the limb blood flow response to cold and cool water immersion. This was to partly ascertain whether a smaller hyperthermic load from a bout of resistance exercise could also blunt the cutaneous response to cold-water and prevent the cold-induced vasodilation reported under resting conditions, i.e. no hyperthermic load (Gregson *et al*., 2011). This is of practical importance since CWI is frequently applied after modes of exercise, which may result in a smaller heat load on the body than examined in Chapter 5 (i.e. endurance exercise). It was shown that FABF and CBF were similarly reduced compared with the CON after both cold (8 °C) and cool water (22 °C) immersion.

These findings were consistent with the observations noted after endurance exercise (Chapter 5) but contrasted with the greater skin blood flow reported in colder water under resting conditions (Gregson *et al*., 2011). Therefore, it is probable that only a small hyperthermic load (core, ~ 0.3 ºC; T_{muscle} 3 cm, ~1 ºC; Tsk_{thigh}, ~0.6 ºC) is required to blunt cutaneous vasoconstrictor responsiveness, prevent cold-induced vasodilation, and lead to similar muscle blood flows between cold and cool water temperatures.

The ~50 % reduction in FABF compared with CON after both cooling conditions was similar to the magnitude of change observed after endurance exercise. These results are also consistent with hemodynamic changes after CWI has been applied after endurance exercise using VOP (Vaile *et al*., 2011; Ihsan *et al*., 2013) and resistance exercise using NIRS (Roberts *et al*, 2015a) methods, respectively. In agreement with the findings in Chapter 5, a greater reduction in T_{muscle} was also noted in the colder water condition. The ~1 ºC difference in deep T_{muscle} observed between cooling conditions suggests that this magnitude of difference also does not directly modify FABF. Consequently, this study indicated that T_{muscle} changes of greater than ~1 ºC may be required to achieve differences in limb blood flow between immersion cooling temperatures and/or cryotherapy modalities. As previously described, the greater decrease in T_{muscle} with colder water temperatures may be more effective in enhancing recovery from EIMD and injury after resistance exercise with cooler temperatures providing a less efficacious recovery option.

In Chapter 7, the most efficacious post-exercise water-cooling strategy ascertained from previous chapters of this thesis (8 ºC for 10 min), was compared against a

typical WBC exposure (-110 ºC for 2min) after cycling exercise. The increased availability of commercial cryotherapy chambers has led to WBC becoming a popular post-exercise recovery method. Whilst WBC can reach sub zero temperatures, the medium of air and water is distinctly different in how cold temperatures are conducted through tissues in the body. However, an absence of research has compared these effects on limb blood flow and thermoregulatory responses between CWI and WBC modalities. The results showed that CWI causes a greater decrease in both FABF (50 %) and CBF compared with WBC. In addition, T_{muscle} was observed to be lower after CWI cooling. These findings suggest that CWI is a better recovery option post-exercise based on potential reductions in muscle blood flow and T_{muscle} enhancing recovery from inflammation and EIMD.

To date, the only other study, which has attempted to compare thermoregulatory responses to CWI and WBC, used a matched duration of cryotherapy exposure (4 min of 8 ºC water immersion vs. 4min of -110 ºC WBC) (Costello *et al*., 2012). In contrast to the to the results presented in Chapter 7, the authors reported that deep T_{muscle} was similarly reduced throughout the post-cooling period after exposure to either CWI or WBC. These findings may be attributed to the different CWI durations used in both studies. Therefore, the use of an ecologically valid CWI duration i.e. 10 min, suggests that the greater CWI cooling duration effectively lowers T_{muscle} to a greater extent compared with a typical WBC exposure.

The results in Chapter 5 and 6 showed that a change in deep T_{muscle} (0.5-1 °C) between different degrees of immersion cooling did not modify FABF. In Chapter 7, limb blood flow was significantly lower during CWI recovery, alongside a

significantly reduced deep T_{muscle} (10 min 1.1 °C and 40 min 1.6 °C). These observations have implications to recovery since it suggests that differences in deep T_{muscle} of >1.0 °C between water temperatures and/or cryotherapy modalities are indeed likely modulate limb blood flow. Therefore, the findings may provide a guideline in order to modify the limb blood flow response to changes in deep tissue temperature to minimize the inflammatory response to EIMD/soft tissue injury.

The results from this thesis may have implications to recovery in clinical settings, (e.g. hospital, physiotherapy and/or rehabilitation facilities), and in athletic settings (e.g., sports clubs/departments or tournament/event providers), to alleviate symptoms of inflammation and EIMD using CWI after different modes of exercise. Current findings indicate that colder water temperatures are more efficacious than cool water temperatures to post-exercise recovery due to greater reductions in T_{muscle} and not muscle blood flow. Additionally, CWI may be a better post-exercise recovery option compared with WBC to reduce inflammation and EIMD due to greater reductions in not only in T_{muscle} but also muscle blood flow.

8.3. LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

In previous work, the effects of different degrees of cooling on both FABF and CBF within the limb were used to provide valid, qualitative, estimates of the effects of cooling on muscle perfusion at rest (Gregson *et al*., 2011). Under these conditions, differences in the direction of change in blood flow in the skin and femoral artery enabled inference with respect to muscle blood flow perfusion. In contrast, in the present investigations (Chapter 5, Chapter 6 & Chapter 7), both FABF and CBF declined with cooling. Because skin blood flow increased before immersion (related to exercise heat stress), decreases in FABF with cooling may reflect decreasing skin blood flow, as opposed to any alterations in muscle perfusion *per se* and the interpretation of these distinct compartmental changes becomes more problematic. The qualitative nature of CBF (Cracowski *et al*., 2006) therefore does not enable direct determination of the true extent to which changes CBF may have influenced muscle perfusion. Future work using methods such as positron-emission tomography (Heinonen *et al*., 2011) to apportion changes in specific domains may permit quantification of post exercise muscle perfusion in response to different degrees of cooling.

In Chapters 5 and 7, the cycling exercise protocols were used to attain a core temperature of 38 °C, prior undertaking the cooling interventions. At this core temperature, the rate of rise in skin blood flow is markedly attenuated despite further significant increases in core temperature (Kellogg *et al*., 1993). This temperature allows for a larger heat exchange between the skin and the cooling exposure compared with lower internal temperatures. However, during the 10 min post-exercise/pre immersion period (prior to cooling exposure) a small drop in core

temperature was recorded, which may have not enabled a maximal heat exchange gradient to occur. It is therefore possible that greater decreases in limb blood flow and/or T_{muscle} may have been observed if core temperature was maintained at 38 °C prior to cooling.

In Chapters 5 & 6, seated rest was used as the control (non-immersion) to compare against the cooling interventions. Whilst seated rest may be representative of a control treatment in a clinical setting, athletes may typically use active recovery (cool-down) in a post-exercise period. Therefore, seated rest may lack efficacy in an athletic context as a control. Similarly, thermoneutral water immersion may be used as a control to assess the hydrostatic pressure effect of water immersion *per se,* on limb blood flow responses. Future work may therefore focus on active recovery or thermoneutral water immersion to compare thermoregulatory and limb blood flow measures against post-exercise cooling.

The water immersion protocols in Chapter 4, 5 & 6 consisted of immersing the legs to the depth of the iliac crest. It remains unknown whether immersing the body to greater depths, i.e. to the xiphoid process or head out immersion, has a similar impact on the limb blood flow response to CWI. Greater thermoneutral immersion depths have been shown to increase $\dot{Q}$, for example, compared to non–immersion, hip-level immersion increases $\dot{Q}$ ~14-29 %, to the xiphoid process ~19-48 % and head out immersion 29-66 % (Wilcock, Cronin & Hing, 2006). Whilst TPR does not change during hip-level thermoneutral immersion (Wilcock Cronin & Hing, 2006), it has been noted to decrease during neck immersion (Gabrielsen *et al*., 2000) suggesting peripheral vasodilation. This decrease is likely to be related to arterial baroreceptor

activation, in particular carotid baroreceptors, in response to an increased CBV from hydrostatic pressure (Connelly *et al*., 1990). Therefore, when the body is exposed to a greater depth of CWI, there may be competition between sympathetic peripheral vasoconstriction from cold-water stimulation and a decrease in TPR (vasodilation) from baroreceptor reflex activity. Consequently, CWI at greater depths may lead to a different limb blood flow response presently observed. This may provide a focus for future research.

WBC Guidelines recommend individuals continuously move their arms and legs inside the cryotherapy chamber during relatively short exposure durations. However, this is problematic in the assessment of limb blood flow due to muscle activation confounding measurements. In Chapter 7, caution was used to select a less severe WBC temperature and duration to limit the prospect of any adverse skin reactions/cold burn injury whilst seated inside the cryotherapy chamber (no adverse skin reactions were noted in the present study) and to match typical durations of WBC protocols. Previous research suggests that colder temperatures e.g. -135 °C may be better for recovery (Selfe *et al*., 2014), therefore colder WBC temperatures and/or longer exposure durations may have a greater impact on deep tissue temperature, which may lead to greater reductions in limb blood flow than presently observed. Further work is required to explain the benefits of lower WBC temperatures and/or increased durations on the limb blood flow response after exercise.

8.4. CONCLUSION

The investigations in Chapters 5 and 6 are the first studies to establish the influence of different degrees of CWI on limb and cutaneous blood flow and thermoregulatory responses after different modes of exercise. In addition, Chapter 7 is also the first study to compare an ecologically valid CWI protocol against WBC on limb and cutaneous blood flows and thermoregulatory responses. It was demonstrated that both cold (8 °C) and cool (22 °C) water immersion leads to potentially similar reductions in muscle blood flow but greater reductions in T_{muscle} after both endurance (cycling) and resistance (squat) exercise. These findings have implications to recovery in sport and rehabilitation contexts since they infer that colder water temperatures are a better recovery selection when applied after these types of exercise by virtue of greater reductions in T_{muscle},. The greater reductions in T_{muscle} may limit secondary muscle injury and attenuate EIMD, potentially leading to enhanced functional and physiological performance. Nonetheless, less noxious water temperatures may offer a suitable alternative to athletes who may not tolerate colder water temperatures. In Chapter 7, the comparison of a widely used CWI and WBC recovery protocol established that CWI is the more efficacious recovery option due to potentially greater reductions in both muscle blood flow and T_{muscle}. These findings suggest that 10 min of CWI is a superior recovery strategy based on key physiological changes (muscle blood flow and deep T_{muscle}.) and should be the preferred modality to enhance recovery from EIMD by both practitioners and athletes.

CHAPTER 9

REFERENCES

ABRAMSON, D. I., CHU, L. S., TUCK, S., JR., LEE, S. W., RICHARDSON, G. & LEVIN, M. 1966. Effect of tissue temperatures and blood flow on motor nerve conduction velocity. *JAMA*, 198, 1082-8.

ALLEN, D. G., WESTERBLAD, H., LEE, J. A. & LANNERGREN, J. 1992. Role of excitation-contraction coupling in muscle fatigue. *Sports Med*, 13, 116-26.

AMENT, W. & VERKERKE, G. J. 2009. Exercise and fatigue. *Sports Med*, 39, 389-422.

ARMSTRONG, R. B., WARREN, G. L. & WARREN, J. A. 1991. Mechanisms of exercise-induced muscle fibre injury. *Sports Med*, 12, 184-207.

ASCENSAO, A., LEITE, M., REBELO, A. N., MAGALHAES, S. & MAGALHAES, J. 2011. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *J Sports Sci*, 29, 217-25.

ATKINSON, G. & NEVILL, A. M. 1998. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med*, 26, 217-38.

ATKINSON, G. & NEVILL, A. M. 2001. Selected issues in the design and analysis of sport performance research. *J Sports Sci*, 19, 811-27.

BAECHLE, T. R. & EARLE, R. W. 2000. *Essentials of strength training and conditioning*, Champaign, Illinois, Human Kinetics.

BAILEY, D. M., ERITH, S. J., GRIFFIN, P. J., DOWSON, A., BREWER, D. S., GANT, N. & WILLIAMS, C. 2007. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *J Sports Sci*, 25, 1163-70.

BAKER, R. J. & BELL, G. W. 1991. The effect of therapeutic modalities on blood flow in the human calf. *J Orthop Sports Phys Ther*, 13, 23-7.

BANFI, G., LOMBARDI, G., COLOMBINI, A. & MELEGATI, G. 2010. Whole-body cryotherapy in athletes. *Sports Med*, 40, 509-17.

BARCROFT, H. & EDHOLM, O. G. 1943. The effect of temperature on blood flow and deep temperature in the human forearm. *J Physiol*, 102, 5-20.

BARNETT, A. 2006. Using recovery modalities between training sessions in elite athletes: does it help? *Sports Med*, 36, 781-96.

BIGLAND-RITCHIE, B. & WOODS, J. J. 1984. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve*, 7, 691-9.

BLEAKLEY, C., MCDONOUGH, S. & MACAULEY, D. 2004. The use of ice in the treatment of acute soft-tissue injury: a systematic review of randomized controlled trials. *Am J Sports Med*, 32, 251-61.

BLEAKLEY, C. M., BIEUZEN, F., DAVISON, G. W. & COSTELLO, J. T. 2014. Whole-body cryotherapy: empirical evidence and theoretical perspectives. *Open Access J Sports Med*, 5, 25-36.

BLEAKLEY, C. M. & DAVISON, G. W. 2010. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med*, 44, 179-87.

BLEAKLEY, C. M. & HOPKINS, J. T. 2010. Is it possible to achieveoptimal levels of tissue cooling in cryotherapy? *Physical Therapy Reviews*, 15, 344-350.

BONDE-PETERSEN, F., SCHULTZ-PEDERSEN, L. & DRAGSTED, N. 1992. Peripheral and central blood flow in man during cold, thermoneutral, and hot water immersion. *Aviat Space Environ Med*, 63, 346-50.

BORG, G. 1970. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*, 2, 92-8.

BOSAK, A., BISHOP, P., SMITH, J., GREEN, M., RICHARDSON, M. & IOSIA, M. 2006. Impact of Cold Water Immersion on 5km Racing Performance. *Medicine and Science in Sports and Exercise*, 38, S233.

BROPHY-WILLIAMS, N., LANDERS, G. & WALLMAN, K. 2011. Effect of immediate and delayed cold water immersion after a high intensity exercise session on subsequent run performance. *J Sports Sci Med*, 10, 665-70.

BUCHHEIT, M., PEIFFER, J. J., ABBISS, C. R. & LAURSEN, P. B. 2009. Effect of cold water immersion on postexercise parasympathetic reactivation. *Am J Physiol Heart Circ Physiol*, 296, H421-7.

BUDGETT, R. 1998. Fatigue and underperformance in athletes: the overtraining syndrome. *Br J Sports Med*, 32, 107-10.

CANNON, B. & NEDERGAARD, J. 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol*, 214, 242-53.

CARNETHON, M. R. & CRAFT, L. L. 2008. Autonomic regulation of the association between exercise and diabetes. *Exerc Sport Sci Rev*, 36, 12-8.

CASEY, D. P., CURRY, T. B. & JOYNER, M. J. 2008. Measuring muscle blood flow: a key link between systemic and regional metabolism. *Curr Opin Clin Nutr Metab Care*, 11, 580-6.

CHARKOUDIAN, N. 2003. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc*, 78, 603-12.

CHEN, T. C., CHEN, H. L., PEARCE, A. J. & NOSAKA, K. 2012. Attenuation of eccentric exercise-induced muscle damage by preconditioning exercises. *Med Sci Sports Exerc*, 44, 2090-8.

CHEUNG, K., HUME, P. & MAXWELL, L. 2003. Delayed onset muscle soreness : treatment strategies and performance factors. *Sports Med*, 33, 145-64.

CLEARY, M. A., SITLER, M. R. & KENDRICK, Z. V. 2006. Dehydration and symptoms of delayed-onset muscle soreness in normothermic men. *J Athl Train*, 41, 36-45.

CONNELLY, T. P., SHELDAHL, L. M., TRISTANI, F. E., LEVANDOSKI, S. G., KALKHOFF, R. K., HOFFMAN, M. D. & KALBFLEISCH, J. H. 1990. Effect of increased central blood volume with water immersion on plasma catecholamines during exercise. *J Appl Physiol*, 69, 651-6.

CONNOLLY, D. A., SAYERS, S. P. & MCHUGH, M. P. 2003. Treatment and prevention of delayed onset muscle soreness. *J Strength Cond Res*, 17, 197-208.

COOKE, J. P. & DZAU, V. J. 1997. Nitric oxide synthase: role in the genesis of vascular disease. *Annu Rev Med*, 48, 489-509.

COSTELLO, J. T., ALGAR, L. A. & DONNELLY, A. E. 2012. Effects of whole-body cryotherapy (-110 degrees C) on proprioception and indices of muscle damage. *Scand J Med Sci Sports*, 22, 190-8.

COSTELLO, J. T., BAKER, P. R., MINETT, G. M., BIEUZEN, F., STEWART, I. B. & BLEAKLEY, C. 2015. Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults. *Cochrane Database Syst Rev*, 9, CD010789.

COSTELLO, J. T., CULLIGAN, K., SELFE, J. & DONNELLY, A. E. 2012a. Muscle, skin and core temperature after -110 degrees c cold air and 8 degrees c water treatment. *PLoS One*, 7, e48190.

COSTELLO, J. T., DONNELLY, A. E., KARKI, A. & SELFE, J. 2014. Effects of whole body cryotherapy and cold water immersion on knee skin temperature. *Int J Sports Med*, 35, 35-40.

COSTELLO, J. T., MCINERNEY, C. D., BLEAKLEY, C. M., SELFE, J. & DONNELLY, A. E. 2012b. The use of thermal imaging in assessing skin temperature following cryotherapy: a review. *Journal of Thermal Biology*, 37, 103-110.

COYLE, E. F. 2000. Physical activity as a metabolic stressor. *Am J Clin Nutr*, 72, 512S-20S.

CRACOWSKI, J. L., MINSON, C. T., SALVAT-MELIS, M. & HALLIWILL, J. R. 2006. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci*, 27, 503-8.

CROWE, M. J., O'CONNOR, D. & RUDD, D. 2007. Cold water recovery reduces anaerobic performance. *Int J Sports Med*, 28, 994-8.

CUI, J., DURAND, S., LEVINE, B. D. & CRANDALL, C. G. 2005. Effect of skin surface cooling on central venous pressure during orthostatic challenge. *Am J Physiol Heart Circ Physiol*, 289, H2429-33.

DAHLSTEDT, A. J. & WESTERBLAD, H. 2001. Inhibition of creatine kinase reduces the rate of fatigue-induced decrease in tetanic [Ca(2+)](i) in mouse skeletal muscle. *J Physiol*, 533, 639-49.

DAVIS, S. L., FADEL, P. J., CUI, J., THOMAS, G. D. & CRANDALL, C. G. 2006. Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress. *J Appl Physiol*, 100, 221-4.

DE GROOT, P. C., POELKENS, F., KOOIJMAN, M. & HOPMAN, M. T. 2004. Preserved flow-mediated dilation in the inactive legs of spinal cord-injured individuals. *Am J Physiol Heart Circ Physiol*, 287, H374-80.

DE OLIVEIRA OTTONE, V., DE CASTRO MAGALHAES, F., DE PAULA, F., AVELAR, N. C., AGUIAR, P. F., DA MATTA SAMPAIO, P. F., DUARTE, T. C., COSTA, K. B., ARAUJO, T. L., COIMBRA, C. C., NAKAMURA, F. Y., AMORIM, F. T. & ROCHA-VIEIRA, E. 2014. The effect of different water immersion temperatures on post-exercise parasympathetic reactivation. *PLoS One*, 9, e113730.

DE ROOS, N. M., BOTS, M. L., SCHOUTEN, E. G. & KATAN, M. B. 2003. Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol*, 29, 401-6.

DE ROOS, N. M., SIEBELINK, E., BOTS, M. L., VAN TOL, A., SCHOUTEN, E. G. & KATAN, M. B. 2002. Trans monounsaturated fatty acids and saturated fatty acids have similar effects on postprandial flow-mediated vasodilation. *Eur J Clin Nutr*, 56, 674-9.

DESCHENES, M. R., KRAEMER, W. J., BUSH, J. A., DOUGHTY, T. A., KIM, D., MULLEN, K. M. & RAMSEY, K. 1998. Biorhythmic influences on functional capacity of human muscle and physiological responses. *Med Sci Sports Exerc*, 30, 1399-407.

DINENNO, F. A., JONES, P. P., SEALS, D. R. & TANAKA, H. 1999. Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. *Circulation*, 100, 164-70.

DOLAN, M. G., THORNTON, R. M., FISH, D. R. & MENDEL, F. C. 1997. Effects of cold water immersion on edema formation after blunt injury to the hind limbs of rats. *J Athl Train*, 32, 233-7.

DOLEZAL, B. A., POTTEIGER, J. A., JACOBSEN, D. J. & BENEDICT, S. H. 2000. Muscle damage and resting metabolic rate after acute resistance exercise with an eccentric overload. *Med Sci Sports Exerc*, 32, 1202-7.

EBBELING, C. B. & CLARKSON, P. M. 1989. Exercise-induced muscle damage and adaptation. *Sports Med*, 7, 207-34.

ENOKA, R. M. & DUCHATEAU, J. 2008. Muscle fatigue: what, why and how it influences muscle function. *J Physiol*, 586, 11-23.

ENWEMEKA, C. S., ALLEN, C., AVILA, P., BINA, J., KONRADE, J. & MUNNS, S. 2002. Soft tissue thermodynamics before, during, and after cold pack therapy. *Med Sci Sports Exerc*, 34, 45-50.

ERIKSEN, M., WAALER, B. A., WALLOE, L. & WESCHE, J. 1990. Dynamics and dimensions of cardiac output changes in humans at the onset and at the end of moderate rhythmic exercise. *J Physiol*, 426, 423-37.

ESTON, R. & PETERS, D. 1999. Effects of cold water immersion on the symptoms of exercise-induced muscle damage. *J Sports Sci*, 17, 231-8.

FABER, J. E. 1988. Effect of local tissue cooling on microvascular smooth muscle and postjunctional alpha 2-adrenoceptors. *Am J Physiol*, 255, H121-30.

FERRARI, M., MOTTOLA, L. & QUARESIMA, V. 2004. Principles, techniques, and limitations of near infrared spectroscopy. *Can J Appl Physiol, 29, 463-87.*

FISCUS, K. A., KAMINSKI, T. W. & POWERS, M. E. 2005. Changes in lower-leg blood flow during warm-, cold-, and contrast-water therapy. *Arch Phys Med Rehabil*, 86, 1404-10.

FITTS, R. H. 1994. Cellular mechanisms of muscle fatigue. *Physiol Rev*, 74, 49-94.

FLOURIS, A. D., WESTWOOD, D. A., MEKJAVIC, I. B. & CHEUNG, S. S. 2008. Effect of body temperature on cold induced vasodilation. *Eur J Appl Physiol*, 104, 491-9.

FONDA, B. & SARABON, N. 2013. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: a crossover study. *Scand J Med Sci Sports*, 23, e270-8.

FRIDEN, J. & LIEBER, R. L. 1992. Structural and mechanical basis of exercise-induced muscle injury. *Med Sci Sports Exerc*, 24, 521-30.

FRYER, M. W., OWEN, V. J., LAMB, G. D. & STEPHENSON, D. G. 1995. Effects of creatine phosphate and P(i) on Ca2+ movements and tension development in rat skinned skeletal muscle fibres. *J Physiol*, 482 (Pt 1), 123-40.

GABRIELSEN, A., VIDEBAEK, R., JOHANSEN, L. B., WARBERG, J., CHRISTENSEN, N. J., PUMP, B. & NORSK, P. 2000. Forearm vascular and neuroendocrine responses to graded water immersion in humans. *Acta Physiol Scand*, 169, 87-94.

GAGNON, D., LEMIRE, B. B., CASA, D. J. & KENNY, G. P. 2010. Cold-water immersion and the treatment of hyperthermia: using 38.6 degrees C as a safe rectal temperature cooling limit. *J Athl Train*, 45, 439-44.

GANDEVIA, S. C., ALLEN, G. M., BUTLER, J. E. & TAYLOR, J. L. 1996. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol*, 490 (Pt 2), 529-36.

GILL, R. W. 1985. Measurement of blood flow by ultrasound: accuracy and sources of error. *Ultrasound Med Biol*, 11, 625-41.

GISSEL, H. 2005. The role of Ca2+ in muscle cell damage. *Ann N Y Acad Sci*, 1066, 166-80.

GREEN, D., CHEETHAM, C., REED, C., DEMBO, L. & O'DRISCOLL, G. 2002. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol*, 93, 361-8.

GREGSON, W., BLACK, M. A., JONES, H., MILSON, J., MORTON, J., DAWSON, B., ATKINSON, G. & GREEN, D. J. 2011. Influence of cold water immersion on limb and cutaneous blood flow at rest. *Am J Sports Med*, 39, 1316-23.

HALSON, S. L., QUOD, M. J., MARTIN, D. T., GARDNER, A. S., EBERT, T. R. & LAURSEN, P. B. 2008. Physiological responses to cold water immersion following cycling in the heat. *Int J Sports Physiol Perform*, 3, 331-46.

HAMLIN, M. J. 2007. The effect of contrast temperature water therapy on repeated sprint performance. *J Sci Med Sport*, 10, 398-402.

HARGREAVES, M. & FEBBRAIO, M. 1998. Limits to exercise performance in the heat. *International Journal of Sports Medicine*, 19, S115-S116.

HARGREAVES, M., MCCONELL, G. & PROIETTO, J. 1995. Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *J Appl Physiol*, 78, 288-92.

HAUSSWIRTH, C., LOUIS, J., BIEUZEN, F., POURNOT, H., FOURNIER, J., FILLIARD, J. R. & BRISSWALTER, J. 2011. Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. *PLoS One*, 6, e27749.

HAUSSWIRTH, C., SCHAAL, K., LE MEUR, Y., BIEUZEN, F., FILLIARD, J. R., VOLONDAT, M. & LOUIS, J. 2013. Parasympathetic activity and blood catecholamine responses following a single partial-body cryostimulation and a whole-body cryostimulation. *PLoS One*, 8, e72658.

HEDGES, L. V. & OLKIN, I. 1985. Statistical methods for meta-analysis, London, Academic Press.

HEINONEN, I., BROTHERS, R. M., KEMPPAINEN, J., KNUUTI, J., KALLIOKOSKI, K. K. & CRANDALL, C. G. 2011. Local heating, but not indirect whole body heating, increases human skeletal muscle blood flow. *J Appl Physiol*, 111, 818-24.

HENSEL, H. & BOMAN, K. K. 1960. Afferent impulses in cutaneous sensory nerves in human subjects. *J Neurophysiol*, 23, 564-78.

HEYMAN, E., B, D. E. G., MERTENS, I. & MEEUSEN, R. 2009. Effects of four recovery methods on repeated maximal rock climbing performance. *Med Sci Sports Exerc*, 41, 1303-10.

HIGGINS, T. R., HEAZLEWOOD, I. T. & CLIMSTEIN, M. 2011. A random control trial of contrast baths and ice baths for recovery during competition in U/20 rugby union. *J Strength Cond Res*, 25, 1046-51.

HIJMERING, M. L., STROES, E. S., PASTERKAMP, G., SIEREVOGEL, M., BANGA, J. D. & RABELINK, T. J. 2001. Variability of flow mediated dilation: consequences for clinical application. *Atherosclerosis*, 157, 369-73.

HODGES, G. J. 2005. Impact of age, gender and exercise on peripheral blood flow. PhD, Liverpool John Moores University.

HODGES, G. J., ZHAO, K., KOSIBA, W. A. & JOHNSON, J. M. 2006. The involvement of nitric oxide in the cutaneous vasoconstrictor response to local cooling in humans. *J Physiol*, 574, 849-57.

HOPKINS, W. G. 2000. Measures of reliability in sports medicine and science. *Sports Med*, 30, 1-15.

HOPKINS, W. G., MARSHALL, S. W., BATTERHAM, A. M. & HANIN, J. 2009. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*, 41, 3-13.

HOWATSON, G., GOODALL, S. & VAN SOMEREN, K. A. 2009. The influence of cold water immersions on adaptation following a single bout of damaging exercise. *Eur J Appl Physiol*, 105, 615-21.

HOWATSON, G., HOAD, M., GOODALL, S., TALLENT, J., BELL, P. G. & FRENCH, D. N. 2012. Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. *J Int Soc Sports Nutr*, 9, 20.

HOWATSON, G. & VAN SOMEREN, K. A. 2008. The prevention and treatment of exercise-induced muscle damage. *Sports Med*, 38, 483-503.

IHSAN, M., WATSON, G. & ABBISS. 2014. PGC-1a mediated muscle aerobic adaptations to exercise, heat and cold exposure. *Cell Mol Exerc Physiol*, 3, e7.

IHSAN, M., WATSON, G. & ABBISS, C. R. 2016. What are the Physiological Mechanisms for Post-Exercise Cold Water Immersion in the Recovery from Prolonged Endurance and Intermittent Exercise? *Sports Med*, 46, 1095-1109.

IHSAN, M., WATSON, G., CHOO, H., LEWANDOWSKI, P., PAPAZZO, S., CAMERON-SMITH, D. & ABISS, C. 2014. Postexercise muscle cooling enhances gene expression of PGC-1a. *Med Sci Sports Exerc*, 46, 1900-1907.

IHSAN, M., WATSON, G., LIPSKI, M. & ABBISS, C. R. 2013. Influence of postexercise cooling on muscle oxygenation and blood volume changes. *Med Sci Sports Exerc*, 45, 876-82.

IMRAY, C., GRIEVE, A. & DHILLON, S. 2009. Cold damage to the extremities: frostbite and non-freezing cold injuries. *Postgrad Med Journal*, 85, 481-488.

IMRAY C., RICHARDS, P., GREEVES , J. & CASTELLANI, J. W. Nonfreezing cold-induced injuries. *J R Army Med Corps*, 157, 79-84.

INGRAM, J., DAWSON, B., GOODMAN, C., WALLMAN, K. & BEILBY, J. 2009. Effect of water immersion methods on post-exercise recovery from simulated team sport exercise. *J Sci Med Sport*, 12, 417-21.

JAKEMAN, J. R., MACRAE, R. & ESTON, R. 2009. A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics*, 52, 456-60.

JENSEN, T. E. & RICHTER, E. A. 2012. Regulation of glucose and glycogen metabolism during and after exercise. *J Physiol*, 590, 1069-76.

JOHANSEN, L. B., BIE, P., WARBERG, J., CHRISTENSEN, N. J. & NORSK, P. 1995. Role of hemodilution on renal responses to water immersion in humans. *Am J Physiol*, 269, R1068-76.

JOHANSEN, L. B., JENSEN, T. U., PUMP, B. & NORSK, P. 1997. Contribution of abdomen and legs to central blood volume expansion in humans during immersion. *J Appl Physiol*, 83, 695-9.

JOHNSON, D. J., MOORE, S., MOORE, J. & OLIVER, R. A. 1979. Effect of cold submersion on intramuscular temperature of the gastrocnemius muscle. *Phys Ther*, 59, 1238-42.

JOO, C., ALLAN, R., DRUST, B., CLOSE, G., JEONG, T., BARTLETT, J., MAHINNEY, C., LOUHELAINEN, J., MORTON, J. & GREGSON, W. Passive and post-exercise cold-water immersion augments PGC-1a and VEGF expression in human skeletal muscle. *Eurn J of Appl Physiol*. [Epub ahead of print] http://dx.doi.org/10.1007/s00421-016-3480-1 [Accessed 20 Oct. 2016].

KARLSSON, J. 1979. Localized muscular fatigue: role of muscle metabolism and substrate depletion. *Exerc Sport Sci Rev*, 7, 1-42.

KARUNAKARA, R. G., LEPHART, S. M. & PINCIVERO, D. M. 1999. Changes in forearm blood flow during single and intermittent cold application. *J Orthop Sports Phys Ther*, 29, 177-80.

KEETON, R. B. & BINDER-MACLEOD, S. A. 2006. Low-frequency fatigue. *Phys Ther*, 86, 1146-50.

KELLER, D. M., SANDER, M., STALLKNECHT, B. & CRANDALL, C. G. 2010. alpha-Adrenergic vasoconstrictor responsiveness is preserved in the heated human leg. *J Physiol,* 588, 3799-808.

KELLOGG JR, D. L. 2006. In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol*, 100, 1709-18.

KELLOGG JR, D. L., JOHNSON, J. M., KENNEY, W. L., PERGOLA, P.E. & KOSIBA, W. A. 1993. Mechanisms of control of skin blood flow during prolonged exercise in humans. *Am J Physiol*, 265, H562-568.

KENT-BRAUN, J. A. 1999. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol Occup Physiol*, 80, 57-63.

KING, M. & DUFFIELD, R. 2009. The effects of recovery interventions on consecutive days of intermittent sprint exercise. *J Strength Cond Res*, 23, 1795-802.

KOGA, S., POOLE, D. C., KONDO, N., OUE, A., OHMAE, E. & BARSTOW, T. J. 2015. Effects of increased skin blood flow on muscle oxygenation/deoxygenation: comparison of time-resolved and continuous-wave near-infrared spectroscopy signals. *Eur J Appl Physiol*, 115, 335-43.

KOURIE, J. I. 1998. Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol*, 275, C1-24.

KREGEL, K. C., SEALS, D. R. & CALLISTER, R. 1992. Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. *J Physiol*, 454, 359-71.

KULIGOWSKI, L. A., LEPHART, S. M., GIANNANTONIO, F. P. & BLANC, R. O. 1998. Effect of whirlpool therapy on the signs and symptoms of delayed-onset muscle soreness. *J Athl Train*, 33, 222-8.

LANE, K. N. & WENGER, H. A. 2004. Effect of selected recovery conditions on performance of repeated bouts of intermittent cycling separated by 24 hours. *J Strength Cond Res*, 18, 855-60.

LASTAYO, P. C., REICH, T. E., URQUHART, M., HOPPELER, H. & LINDSTEDT, S. L. 1999. Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. *Am J Physiol*, 276, R611-5.

LAYZER, R. B. 1990. Muscle metabolism during fatigue and work. *Baillieres Clin Endocrinol Metab*, 4, 441-59.

LEE, D. T., TONER, M. M., MCARDLE, W. D., VRABAS, I. S. & PANDOLF, K. B. 1997. Thermal and metabolic responses to cold-water immersion at knee, hip, and shoulder levels. *J Appl Physiol*, 82, 1523-1530.

LEE, H., NATSUI, H., AKIMOTO, T., YANAGI, K., OHSHIMA, N. & KONO, I. 2005. Effects of cryotherapy after contusion using real-time intravital microscopy. *Med Sci Sports Exerc*, 37, 1093-8.

LEEDER, J., GISSANE, C., VAN SOMEREN, K., GREGSON, W. & HOWATSON, G. 2012. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med*, 46, 233-40.

LIN, Y. C. 1984. Circulatory functions during immersion and breath-hold dives in humans. *Undersea Biomed Res*, 11, 123-38.

LOMBARDI, G., LANTERI, P., PORCELLI, S., MAURI, C., COLOMBINI, A., GRASSO, D., ZANI, V., BONOMI, F. G., MELEGATI, G. & BANFI, G. 2013. Hematological profile and martial status in rugby players during whole body cryostimulation. *PLoS One*, 8, e55803.

LOVALLO, W. 1975. The cold pressor test and autonomic function: a review and integration. *Psychophysiology*, 12, 268-82.

MACDOUGALL, J. D., MCKELVIE, R. S., MOROZ, D. E., SALE, D. G., MCCARTNEY, N. & BUICK, F. 1992. Factors affecting blood pressure during heavy weight lifting and static contractions. *J Appl Physiol*, 73, 1590-7.

MCDERMOTT, M. M., LIU, K., CARROLL, T. J., TIAN, L., FERRUCCI, L., LI, D., CARR, J., GURALNIK, J. M., KIBBE, M., PEARCE, W. H., YUAN, C., MCCARTHY, W., KRAMER, C. M., TAO, H., LIAO, Y., CLARK, E. T., XU, D., BERRY, J., OROZCO, J., SHARMA, L. & CRIQUI, M. H. 2011. Superficial femoral artery plaque and functional performance in peripheral arterial disease: walking and leg circulation study (WALCS III). *JACC Cardiovasc Imaging*, 4, 730-9.

MCGORM, H., ROBERTS, L. A. & PEAKE, J. M. 2015. Cold water immersion; practices, trends and avenues of effect. *Aspetar Sports Medicine Journal*, 4, 106-111.

MCKENZIE, M. A., GREENLEAF, J. E., LOOFT-WILSON, R. & BARNES, P. R. 1999. Leucocytosis, thrombocytosis, and plasma osmolality during rest and exercise: a hypothesis. *J Physiol Pharmacol*, 50, 259-73.

MEEUSEN, R. & LIEVENS, P. 1986. The use of cryotherapy in sports injuries. *Sports Med*, 3, 398-414.

MEIRELLES C. M., LEITE, S. P., MONTENEGRO, C. A. & GOMES, P. S. 2007. Reliability of brachial artery flow-mediated dilatation measurement using ultrasound. *Arq Bras Cardiol*, 89, 160-7, 176-83.

MERRICK, M. A. 2002. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train*, 37, 209-17.

MERRICK, M. A., JUTTE, L. S. & SMITH, M. E. 2003. Cold Modalities With Different Thermodynamic Properties Produce Different Surface and Intramuscular Temperatures. *J Athl Train*, 38, 28-33.

MERRICK, M. A. & MCBRIER, N. M. 2010. Progression of secondary injury after musculoskeletal trauma-a window of opportunity? *J Sport Rehabil*, 19, 380-8.

MERRICK, M. A., RANKIN, J. M., ANDRES, F. A. & HINMAN, C. L. 1999. A preliminary examination of cryotherapy and secondary injury in skeletal muscle. *Med Sci Sports Exerc*, 31, 1516-21.

MILA-KIERZENKOWSKA, C., WOZNIAK, A., WOZNIAK, B., DREWA, G., RAKOWSKI, A., JURECKA, A. & RAJEWSKI, R. 2009. Whole-body cryostimulation in kayaker women: a study of the effect of cryogenic temperatures on oxidative stress after the exercise. *J Sports Med Phys Fitness*, 49, 201-7.

MILLER, E., MARKIEWICZ, L., SALUK, J. & MAJSTEREK, I. 2012. Effect of short-term cryostimulation on antioxidative status and its clinical applications in humans. *Eur J Appl Physiol*, 112, 1645-52.

MONTGOMERY, P. G., PYNE, D. B., HOPKINS, W. G., DORMAN, J. C., COOK, K. & MINAHAN, C. L. 2008. The effect of recovery strategies on physical performance and cumulative fatigue in competitive basketball. *J Sports Sci*, 26, 1135-45.

MORGAN, D. L. & ALLEN, D. G. 1999. Early events in stretch-induced muscle damage. *J Appl Physiol*, 87, 2007-15.

MORRISON, S. F., NAKAMURA, K. & MADDEN, C. J. 2008. Central control of thermogenesis in mammals. *Exp Physiol*, 93, 773-97.

MORTENSEN, S. P., DAMSGAARD, R., DAWSON, E. A., SECHER, N. H. & GONZALEZ-ALONSO, J. 2008. Restrictions in systemic and locomotor skeletal muscle perfusion, oxygen supply and VO2 during high-intensity whole-body exercise in humans. *J Physiol*, 586, 2621-35.

MOUROT, L., BOUHADDI, M. & REGNARD, J. 2009. Effects of the cold pressor test on cardiac autonomic control in normal subjects. *Physiol Res*, 58, 83-91.

MYRER, J. W., MEASOM, G. & FELLINGHAM, G. W. 1998. Temperature changes in the human leg during and after two methods of cryotherapy. *J Athl Train*, 33, 25-9.

NAKAMURA, K. & MORRISON, S. F. 2011. Central efferent pathways for cold-defensive and febrile shivering. *J Physiol*, 589, 3641-58.

NELSON, T. R. & PRETORIUS, D. H. 1988. The Doppler signal: where does it come from and what does it mean? *AJR Am J Roentgenol*, 151, 439-47.

NEVILL, A. M. & ATKINSON, G. 1997. Assessing agreement between measurements recorded on a ratio scale in sports medicine and sports science. *Br J Sports Med*, 31, 314-8.

NEWSHOLME, E. A. A., I. BLOMSTRAND, E. 1987. Amino acids, brain neurotransmittersand a functional link between muscle and brain that is important in sustained exercise. In: BENZI, G. (ed.) *Advances in Myochemistry*. London: John Libbey Eurotext.

NIELSEN, J. J., MOHR, M., KLARSKOV, C., KRISTENSEN, M., KRUSTRUP, P., JUEL, C. & BANGSBO, J. 2004. Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *J Physiol*, 554, 857-70.

OLIVE, J. L., McCULLY, K. L. & DUDLEY, G. A. 2002. Blood flow response in individuals with incomplete spinal cord injuries. *Spinal Cord*, 40, 639-645.

ORTENBLAD, N., WESTERBLAD, H. & NIELSEN, J. 2013. Muscle glycogen stores and fatigue. *J Physiol*-London, 591, 4405-4413.

PADDON-JONES, D. J. & QUIGLEY, B. M. 1997. Effect of cryotherapy on muscle soreness and strength following eccentric exercise. *Int J Sports Med*, 18, 588-93.

PARK, K. S., CHOI, J. K. & PARK, Y. S. 1999. Cardiovascular regulation during water immersion. *Appl Human Sci*, 18, 233-41.

PAROUTY, J., AL HADDAD, H., QUOD, M., LEPRETRE, P. M., AHMAIDI, S. & BUCHHEIT, M. 2010. Effect of cold water immersion on 100-m sprint performance in well-trained swimmers. *Eur J Appl Physiol*, 109, 483-90.

PEAKE, J. M., ROBERTS, L. A., FIGUEIREDO, V. C., EGNER, I., KROG, S., AAS, S.N., SUZUKI, K., MARKWORTH, J. F., COOMBES, J. S., CAMERON-SMITH, D. & RAASTAD, T. 2016. The effects of cold water immersion and active recovery on inflammation and cell stress responses in human skeletal muscle after resistance exercise. *J Physiol*, [Epub ahead of print] http://dx.doi.org/ 10.1113/JP272881 [Accessed 20 Oct. 2016].

PEIFFER, J. J., ABBISS, C. R., NOSAKA, K., PEAKE, J. M. & LAURSEN, P. B. 2009a. Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. *J Sci Med Sport*, 12, 91-6.

PEIFFER, J. J., ABBISS, C. R., WATSON, G., NOSAKA, K. & LAURSEN, P. B. 2009b. Effect of cold-water immersion duration on body temperature and muscle function. *J Sports Sci*, 27, 987-93.

PEIFFER, J. J., ABBISS, C. R., WATSON, G., NOSAKA, K. & LAURSEN, P. B. 2010a. Effect of a 5-min cold-water immersion recovery on exercise performance in the heat. *Br J Sports Med*, 44, 461-5.

PEIFFER, J. J., ABBISS, C. R., WATSON, G., NOSAKA, K. & LAURSEN, P. B. 2010b. Effect of cold water immersion on repeated 1-km cycling performance in the heat. *J Sci Med Sport*, 13, 112-6.

PERIMED. 2015. Laser Doppler monitoring. Available at: http:// perimed-instruments.com [Online] [Accessed 20th December 2015].

POINTON, M., DUFFIELD, R., CANNON, J. & MARINO, F. E. 2012. Cold water immersion recovery following intermittent-sprint exercise in the heat. *Eur J Appl Physiol*, 112, 2483-94.

POPPENDIECK, W., FAUDE, O., WEGMANN, M. & MEYER, T. 2013. Cooling and performance recovery of trained athletes: a meta-analytical review. *Int J Sports Physiol Perform*, 8, 227-42.

POURNOT, H., BIEUZEN, F., DUFFIELD, R., LEPRETRE, P. M., COZZOLINO, C. & HAUSSWIRTH, C. 2011a. Short term effects of various water immersions on recovery from exhaustive intermittent exercise. *Eur J Appl Physiol*, 111, 1287-95.

POURNOT, H., BIEUZEN, F., LOUIS, J., MOUNIER, R., FILLARD, J. R., BARBICHE, E. & HAUSSWIRTH, C. 2011b. Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. *PLoS One*, 6, e22748.

POWERS, S. K. & JACKSON, M. J. 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*, 88, 1243-76.

PROSKE, U. & MORGAN, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol*, 537, 333-45.

PROULX, C. I., DUCHARME, M. B. & KENNY, G. P. 2003. Effect of water temperature on cooling efficiency during hyperthermia in humans. *J Appl Physiol*, 94, 1317-23.

RADEGRAN, G. 1999. Limb and skeletal muscle blood flow measurements at rest and during exercise in human subjects. *Proc Nutr Soc*, 58, 887-98.

RAMANATHAN, N. L. 1964. A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol*, 19, 531-3.

RAVEN, P. B., WILKERSON, J. E., HORVATH, S. M. & BOLDUAN, N. W. 1975. Thermal, metabolic, and cardiovascular responses to various degrees of cold stress. *Can J Physiol Pharmacol*, 53, 293-8.

REILLY, T. & BROOKS, G. A. 1990. Selective persistence of circadian rhythms in physiological responses to exercise. *Chronobiol Int*, 7, 59-67.

ROBERTS, D. H., TSAO, Y. & BRECKENRIDGE, A. M. 1986. The reproducibility of limb blood flow measurements in human volunteers at rest and after exercise by using mercury-in-Silastic strain gauge plethysmography under standardized conditions. *Clin Sci (Lond)*, 70, 635-8.

ROBERTS, L. A., MUTHALIB, M., STANLEY, J., LICHTWARK, G., NOSAKA, K., COOMBES, J. S. & PEAKE, J. M. 2015a. Effects of cold water immersion and active recovery on hemodynamics and recovery of muscle strength following resistance exercise. *Am J Physiol Regul Integr Comp Physiol*, 309, R389-98.

ROBERTS, L. A., NOSAKA, K., COOMBES, J. S. & PEAKE, J. M. 2014. Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. *Am J Physiol Regul Integr Comp Physiol*, 307, R998-R1008.

ROBERTS, L. A., RAASTAD, T., MARKWORTH, J. F., FIGUEIREDO, V. C., EGNER, I. M., SHIELD, A., CAMERON-SMITH, D., & COOMBES, J. S. 2015b. Post-exercise cold water immersion attenuates acute anabolic signaling and long-term adaptations in muscle to strength training. *J Physiol*, 18, 4285-4301.

ROBEY, E., DAWSON, B., HALSON, S., GOODMAN, C., GREGSON, W. & EASTWOOD, P. 2013. Post-exercise cold water immersion: effect on core temperature and melatonin responses. *Eur J Appl Physiol*, 113, 305-11.

ROELANDS, B. & MEEUSEN, R. 2010. Alterations in central fatigue by pharmacological manipulations of neurotransmitters in normal and high ambient temperature. *Sports Med*, 40, 229-46.

ROWSELL, G. J., COUTTS, A. J., REABURN, P. & HILL-HAAS, S. 2009. Effects of cold-water immersion on physical performance between successive matches in high-performance junior male soccer players. *J Sports Sci*, 27, 565-73.

ROWSELL, G. J., COUTTS, A. J., REABURN, P. & HILL-HAAS, S. 2011. Effect of post-match cold-water immersion on subsequent match running performance in junior soccer players during tournament play. *J Sports Sci*, 29, 1-6.

SACHDEV, S. & DAVIES, K. J. 2008. Production, detection, and adaptive responses to free radicals in exercise. *Free Radic Biol Med*, 44, 215-23.

SCHAAL, K., LE MEUR, Y., BIEUZEN, F., PETIT, O., HELLARD, P., TOUSSAINT, J. F. & HAUSSWIRTH, C. 2013. Effect of recovery mode on postexercise vagal reactivation in elite synchronized swimmers. *Appl Physiol Nutr Metab*, 38, 126-33.

SCHEPERS, R. J. & RINGKAMP, M. 2010. Thermoreceptors and thermosensitive afferents. *Neurosci Biobehav Rev*, 34, 177-84.

SCHNIEPP, J., CAMPBELL, T. S., POWELL, K. L. & PINCIVERO, D. M. 2002. The effects of cold-water immersion on power output and heart rate in elite cyclists. *J Strength Cond Res*, 16, 561-6.

SELFE, J., ALEXANDER, J., COSTELLO, J. T., MAY, K., GARRATT, N., ATKINS, S., DILLON, S., HURST, H., DAVISON, M., PRZYBYLA, D., COLEY, A., BITCON, M., LITTLER, G. & RICHARDS, J. 2014. The effect of three different (-135 degrees C) whole body cryotherapy exposure durations on elite rugby league players. *PLoS One*, 9, e86420.

SELKOW, N. M., PIETROSIMONE, B. G. & SALIBA, S. A. 2011. Subcutaneous thigh fat assessment: a comparison of skinfold calipers and ultrasound imaging. *J Athl Train*, 46, 50-4.

SELLWOOD, K. L., BRUKNER, P., WILLIAMS, D., NICOL, A. & HINMAN, R. 2007. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br J Sports Med*, 41, 392-7.

SENDOWSKI, I., SAVOUREY, G., BESNARD, Y. & BITTEL, J. 1997. Cold induced vasodilatation and cardiovascular responses in humans during cold water immersion of various upper limb areas. *Eur J Appl Physiol Occup Physiol*, 75, 471-7.

SHOEMAKER, J. K., POZEG, Z. I. & HUGHSON, R. L. 1996. Forearm blood flow by Doppler ultrasound during test and exercise: tests of day-to-day repeatability. *Med Sci Sports Exerc*, 28, 1144-9.

SJODIN, B., HELLSTEN WESTING, Y. & APPLE, F. S. 1990. Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Med*, 10, 236-54.

SMITH, J. L., MARTIN, P. G., GANDEVIA, S. C. & TAYLOR, J. L. 2007. Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *Journal of Applied Physiology*, 103, 560-568.

SMITH, L. L. 1991. Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med Sci Sports Exerc*, 23, 542-51.

SOGAARD, K., GANDEVIA, S. C., TODD, G., PETERSEN, N. T. & TAYLOR, J. L. 2006. The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol*, 573, 511-23.

SRAMEK, P., SIMECKOVA, M., JANSKY, L., SAVLIKOVA, J. & VYBIRAL, S. 2000. Human physiological responses to immersion into water of different temperatures. *Eur J Appl Physiol*, 81, 436-42.

STACEY, D. L., GIBALA, M. J., MARTIN GINIS, K. A. & TIMMONS, B. W. 2010. Effects of recovery method after exercise on performance, immune changes, and psychological outcomes. *J Orthop Sports Phys Ther*, 40, 656-65.

STANLEY, J., BUCHHEIT, M. & PEAKE, J. M. 2012. The effect of post-exercise hydrotherapy on subsequent exercise performance and heart rate variability. *Eur J Appl Physiol*, 112, 951-61.

STANLEY, J., PEAKE, J. M. & BUCHHEIT, M. 2013. Consecutive days of cold water immersion: effects on cycling performance and heart rate variability. *Eur J Appl Physiol*, 113, 371-84.

STANLEY, J., PEAKE, J. M., COOMBES, J. S. & BUCHHEIT, M. 2014. Central and peripheral adjustments during high-intensity exercise following cold water immersion. *Eur J Appl Physiol*, 114, 147-63.

STEPHENS, J. M., HALSON, S., MILLER, J., SLATER, G. J. &ASKEW, C. D. 2016. Cold water immersion for athletic recovery: one size does not fit all. *Int J Sports Physiol Perform*, 1-24 [Epub ahead of print]: http://dx.doi.org/10.1123/ijspp.2016--0095 [Accessed 20th October 2016].

STOCKS, J. M., PATTERSON, M. J., HYDE, D. E., JENKINS, A. B., MITTLEMAN, K. D. & TAYLOR, N. A. 2004. Effects of immersion water temperature on whole-body fluid distribution in humans. *Acta Physiol Scand*, 182, 3-10.

TABER, C., CONTRYMAN, K., FAHRENBRUCH, J., LACOUNT, K. & CORNWALL, M. W. 1992. Measurement of reactive vasodilation during cold gel pack application to nontraumatized ankles. *Phys Ther*, 72, 294-9.

TAYLOR, J. L., AMANN, M., DUCHATEAU, J., MEEUSEN, R. & RICE, C. L. 2016. Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again. *Med Sci Sports Exerc*. 48, 2294-2306.

TAYLOR, J. L. & GANDEVIA, S. C. 2008. A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol.*, 104, 542-550.

TEE, J. C., BOSCH, A. N. & LAMBERT, M. I. 2007. Metabolic consequences of exercise-induced muscle damage. *Sports Med*, 37, 827-36.

THIJSSEN, D. H., BLACK, M. A., PYKE, K. E., PADILLA, J., ATKINSON, G., HARRIS, R. A., PARKER, B., WIDLANSKY, M. E., TSCHAKOVSKY, M. E. & GREEN, D. J. 2011. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol*, 300, H2-12.

THOMPSON, D., NICHOLAS, C. W. & WILLIAMS, C. 1999. Muscular soreness following prolonged intermittent high-intensity shuttle running. *Journal of Sports Sciences*, 17, 387-395.

THORLACIUS, H., VOLLMAR, B., WESTERMANN, S., TORKVIST, L. & MENGER, M. D. 1998. Effects of local cooling on microvascular hemodynamics and leukocyte adhesion in the striated muscle of hamsters. *J Trauma*, 45, 715-9.

THORSSON, O., LILJA, B., AHLGREN, L., HEMDAL, B. & WESTLIN, N. 1985. The effect of local cold application on intramuscular blood flow at rest and after running. *Med Sci Sports Exerc*, 17, 710-3.

THRUSH, A. & HARTSHONE, T. 2005. *Peripheral vascular ultrasound: how, why and when*, Edinburgh, Elsevier Churchill Livingstone.

TIPTON, M. J. 1989. The initial responses to cold-water immersion in man. *Clin Sci (Lond)*, 77, 581-8.

TODD, G., TAYLOR, J. L. & GANDEVIA, S. C. 2003. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol*, 551, 661-71.

TSCHAKOVSKY, M. E., SUJIRATTANAWIMOL, K., RUBLE, S. B., VALIC, Z. & JOYNER, M. J. 2002. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J Physiol*, 541, 623-35.

VAILE, J., HALSON, S., GILL, N. & DAWSON, B. 2008. Effect of hydrotherapy on recovery from fatigue. *Int J Sports Med*, 29, 539-44.

VAILE, J., O'HAGAN, C., STEFANOVIC, B., WALKER, M., GILL, N. & ASKEW, C. D. 2011. Effect of cold water immersion on repeated cycling performance and limb blood flow. *Br J Sports Med*, 45, 825-9.

VERSEY, N. G., HALSON, S. L. & DAWSON, B. T. 2013. Water immersion recovery for athletes: effect on exercise performance and practical recommendations. *Sports Med*, 43, 1101-30.

VEZZOLI, A., PUGLIESE, L., MARZORATI, M., SERPIELLO, F. R., LA TORRE, A. & PORCELLI, S. 2014. Time-course changes of oxidative stress response to high-intensity discontinuous training versus moderate-intensity continuous training in masters runners. *PLoS One*, 9, e87506.

VICTOR, R. G., LEIMBACH, W. N., JR., SEALS, D. R., WALLIN, B. G. & MARK, A. L. 1987. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension*, 9, 429-36.

VOLLAARD, N. B., SHEARMAN, J. P. & COOPER, C. E. 2005. Exercise-induced oxidative stress:myths, realities and physiological relevance. *Sports Med*, 35, 1045-62.

WAKABAYASHI, H., HANAI, A., YOKOYAMA, S. & NOMURA, T. 2006. Thermal insulation and body temperature wearing a thermal swimsuit during water immersion. *J Physiol Anthropol*, 25, 331-8.

WARREN, G. L., INGALLS, C. P., LOWE, D. A. & ARMSTRONG, R. B. 2001. Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exerc Sport Sci Rev*, 29, 82-7.

WEBB, P. 1986. Afterdrop of body temperature during rewarming: an alternative explanation. J Appl Physiol, 60, 385-90.

WESTERBLAD, H., ALLEN, D. G. & LANNERGREN, J. 2002. Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci*, 17, 17-21.

WESTERLUND, T., OKSA, J., SMOLANDER, J. & MIKKELSSON, M. 2003. Thermal responses during and after whole-body cryotherapy (-110 degrees C). *Journal of Thermal Biology*, 28, 601-608.

WHITE, G. E. & WELLS, G. D. 2013. Cold-water immersion and other forms of cryotherapy: physiological changes potentially affecting recovery from high-intensity exercise. *Extrem Physiol Med*, 2, 26.

WILCOCK, I. M., CRONIN, J. B. & HING, W. A. 2006. Physiological response to water immersion: a method for sport recovery? *Sports Med*, 36, 747-65.

WILSON, T. E., CUI, J. & CRANDALL, C. G. 2002. Effect of whole-body and local heating on cutaneous vasoconstrictor responses in humans. *Auton Neurosci*, 97, 122-8.

WILSON, T. E., SAUDER, C. L., KEARNEY, M. L., KUIPERS, N. T., LEUENBERGER, U. A., MONAHAN, K. D. & RAY, C. A. 2007. Skin-surface

cooling elicits peripheral and visceral vasoconstriction in humans. *J Appl Physiol*, 103, 1257-62.

WISSLER, E. H. 2008. A quantitative assessment of skin blood flow in humans. *Eur J Appl Physiol*, 104, 145-57.

WOLF, S. & HARDY, J. D. 1941. Studies on Pain. Observations on Pain Due to Local Cooling and on Factors Involved in the "Cold Pressor" Effect. *J Clin Invest*, 20, 521-33.

WOODMAN, R. J., PLAYFORD, D. A., WATTS, G. F., CHEETHAM, C., REED, C., TAYLOR, R. R., PUDDEY, I. B., BEILIN, L. J., BURKE, V., MORI, T. A. & GREEN, D. 2001. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol*, 91, 929-37.

WRAY, D. W., DONATO, A. J., NISHIYAMA, S. K. & RICHARDSON, R. S. 2007. Acute sympathetic vasoconstriction at rest and during dynamic exercise in cyclists and sedentary humans. *J Appl Physiol*, 102, 704-12.

YAMANE, M., TERUYA, H., NAKANO, M., OGAI, R., OHNISHI, N. & KOSAKA, M. 2006. Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation. *Eur J Appl Physiol*, 96, 572-80.

YANAGISAWA, O., HOMMA, T., OKUWAKI, T., SHIMAO, D. & TAKAHASHI, H. 2007. Effects of cooling on human skin and skeletal muscle. *Eur J Appl Physiol*, 100, 737-45.

YANAGISAWA, O., KUDO, H., TAKAHASHI, N. & YOSHIOKA, H. 2004. Magnetic resonance imaging evaluation of cooling on blood flow and oedema in skeletal muscles after exercise. *Eur J Appl Physiol*, 91, 737-40.

YANAGISAWA, O., NIITSU, M., TAKAHASHI, H., GOTO, K. & ITAI, Y. 2003. Evaluations of cooling exercised muscle with MR imaging and 31P MR spectroscopy. *Med Sci Sports Exerc*, 35, 1517-23.

YEARGIN, S. W., CASA, D. J., MCCLUNG, J. M., KNIGHT, J. C., HEALEY, J. C., GOSS, P. J., HARVARD, W. R. & HIPP, G. R. 2006. Body cooling between two bouts of exercise in the heat enhances subsequent performance. *J Strength Cond Res*, 20, 383-9.

YOUNG, A. J., SAWKA, M. N., EPSTEIN, Y., DECRISTOFANO, B. & PANDOLF, K. B. 1987. Cooling different body surfaces during upper and lower body exercise. *J Appl Physiol*, 63, 1218-23.

ZIEMANN, E., OLEK, R. A., KUJACH, S., GRZYWACZ, T., ANTOSIEWICZ, J., GARSZTKA, T. & LASKOWSKI, R. 2012. Five-day whole-body cryostimulation, blood inflammatory markers, and performance in high-ranking professional tennis players. *J Athl Train*, 47, 664-72.

APPENDIX 1

CRYOTHERAPY SUBJECT SCREENING FORM

Tom Reilly Building
Liverpool John Moores University

To help us ensure your safety and well being, please answer the following questions.

1. Have you ever suffered from any of the following cold related conditions?

    - Raynaud's disease
    - Vasospastic diseases (blood vessels don't dilate properly)
    - Cold hypersensitivity
    - Compromised local circulation

    No ☐ Yes ☐

2. Have you ever had a negative or allergic reaction to cryotherapy (cold) exposure? ☐ ☐

    No Yes

3. Has your doctor ever said you have a heart condition?

    No ☐ Yes ☐

4. Do you currently have or ever been diagnosed with high blood pressure?

    No ☐ Yes ☐

5. Have you been diagnosed with a rheumatoid condition?

    No ☐ Yes ☐

6. Have you been diagnosed with a respiratory condition e.g. asthma?

    No ☐ Yes ☐

Subject name (print):_____
Subject signature:_____
Date:_____
Signature of person conducting assessment:_____

APPENDIX 2

School of Sport & Exercise Sciences

Participant Information Sheet

Project Title: The effect of cold-water immersion on the thermoregulatory and vascular responses during recovery from exercise

Research Team: Mr. Chris Mawhinney
Dr. Warren Gregson
Dr. Helen Jones
Prof. Danny Green
Dr David Low

General Guidance

The purpose of this form is to provide you with all the necessary information relating to the investigation that you are about to undertake. It is important that you read it all carefully and understand the procedures involved before signing. Any questions relating to the procedures will be answered on request. This form acknowledges your acceptance of the test conditions.

1. What is the purpose of the study?

The stress associated with demanding exercise often temporarily impairs an athlete's performance. This impairment may be acute, lasting minutes or hours, or more long-term if exercise induced muscle injury and related delayed onset muscle soreness (DOMS) are prevalent. In an attempt to enhance the process of recovery following demanding exercise, various strategies are frequently adopted (e.g. nutrition, stretching and sleep). More recently, cold water immersion has been widely used in the recovery process despite little scientific evidence to support its use. Therefore, the purpose of this study is to determine the effect of cold water immersion on muscle and skin blood flow following exercise.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free

to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights/any future treatment/service you receive.

3. What will happen to me if I take part?

You will be required to attend the LJMU Tom Reilly Building laboratory on 4 separate occasions. **Prior to arrival** at the laboratory you will have refrained from exercise, alcohol, tobacco and caffeine for a 48-hour period. You will be required to wear your normal training clothing. Prior to visit 2 you will be required to consume and record your normal daily food intake over the 24 h preceding your arrival at the laboratory. This food intake will be replicated on visits 3 and 4.

Visit 1. (Approx 1.5 h in duration)

- **On arrival.** Completion of a medical questionnaire will determine your suitability as a subject for the investigation. This will reduce the possibilities of any risks or discomforts that you may experience as a result of the procedures involved in this investigation.
- **Measurement of Body composition.** After you have successfully finished the questionnaire an evaluation of your body size, shape and composition will be completed. Measurements taken will include body mass (kg) and height (m) recorded with a calibrated measuring device and scales. This procedure results in no discomfort whatsoever. All measurements will be taken in private.
- **Maximal Exercise Capacity Test**. You will complete a test to determine your maximal exercise capacity. Such tests are carried out by experienced personnel in our laboratory on a daily basis and represent little more discomfort to you than is experienced during your normal training. You will be required to perform incremental cycle exercise on a stationary cycle ergometer until volitional exhaustion. This test begins with an initial 5-min warm-up at 120 W followed by a 2-min stage at 180 W with an increase of 30 W every 2-min thereafter until you can no longer continue. The air that you breathe out during exercise will be collected via a small face mask worn throughout the test. The gas will be measured continuously using an online

gas analysis system connected to the mask. Your heart rate will also be measured via a loose fitting band strapped around your chest. The duration of the maximal exercise capacity test should be between 10 and 15-min.

- **Familiarization session**. General procedures for muscle temperature measurement and insertion of the rectal probe will be demonstrated by verbal explanation. The final part of the session will familiarize you with the cold-water immersion procedures. This will take place in a simple water storage unit located within the laboratory and will simply require your legs to be fully immersed in 8 ^{o}C water, whilst seated on a retractable bed. This immersion is used in the familiarization session to ensure all participants can tolerate the water temperature. Minimal discomfort will be felt during immersion.

<u>Visits 2, 3 and 4</u>. Exercise protocol and treatment (each visit will be approximately 3 hours in duration). Please bring a spare pair of shorts and a towel with you to these visits.

At each visit you will be asked to complete a continuous sub-maximal cycle protocol until you reach an internal body temperature of 38 °C by cycling at 70% of your maximal exercise capacity. During exercise, internal body temperature will be continuously monitored via a rectal probe, which you will be asked to insert prior to exercise to a depth of 10-15 cm beyond the external anal sphincter. The assessment of rectal temperature is a routine procedure and should not result in more than a small amount of discomfort. Heart rate will also be continuously measured every 5 s using a short-range telemetry system via a loose fitting chest belt.

Following exercise you will be asked to complete a different post-exercise testing protocol at each of the 3 visits. Which post-exercise protocol you will be asked to undertake at each visit will be randomly determined. The post-exercise protocols are:

- Immersion in **8 °C** water to the level of the hips for a period of 10-min.
- Immersion in **22 °C** water to the level of the hips for a period of 10-min.

- Seated in a semi-reclined position for a period of 10-min (no water immersion).

Immediately prior, during and for up to 30 minutes following the post-exercise testing protocol the following will be measured:

- Oxygen uptake will be assessed using an online gas analysis system connected to a face mask.
- Muscle temperature will be monitored via a small needle inserted to a depth of 3-4cm into the lateral side of the upper thigh. The assessment of muscle temperature is a routine procedure and should not result in more than a small amount of discomfort.
- Leg blood flow will be measured from the femoral artery using non-invasive duplex-Doppler ultrasound.
- Skin temperature will be measured via placement of non-invasive skin thermistors attached on the chest, forearm, thigh and calf using surgical tape.
- Skin blood flow will be measured using a non-invasive laser Doppler probe. Laser Doppler probes are attached to the forearm and foot using double sided sticky tape.
- Internal body temperature will be measured using the self inserted rectal thermometer.

4. Are there any risks/benefits involved?

- **Risks**

Even though it will be extremely rare, insertion of the muscle temperature needle may cause fainting. A risk of cross-contamination can occur following the use of a muscle temperature needle. To minimize any risk, only sterilized needles are used, with a new needle used for each measurement. The use of surgical gloves and sterilization of the puncture site will reduce the risk of an infection. A waterproof dressing will be applied to the area when you're required to undergo water immersion. Although extremely rare, a risk of cross-contamination can occur following insertion of a rectal probe. This risk is minimized by cleaning all rectal probes with a sterilization solution following their use and using surgical gloves during insertion of the probe. Although

problems with cryotherapy are rare, there are contraindications to its use. Contraindications include Raynaud's or other vasospastic diseases, cold hypersensitivity, cardiac disorders, compromised local circulation and rheumatoid conditions. You will be initially screened for the above contraindications prior to taking part in this study via answering questions on a screening form. Any identified contraindications to the application of cryotherapy will result in your exclusion from this study.

- **Benefits**

You will receive information on your current level of aerobic fitness and physiological responses to cycle exercise and cold-water immersion.

5. Will my taking part in the study be kept confidential?

The data collected in this investigation may be reported in presentations at national and/or international conferences and/or in journal publications. Personal information will however be treated in the strictest confidence with no association made between your identity and the data observed. In order to protect subject anonymity individual subject codes will be used to identify each subject's data when recording and storing any raw data on computers, data storage units or on paper. Only the principal investigator will have access to the coding system.

NB. You must freely volunteer to be a subject and are able to withdraw, without prejudice, at any time. Thank you for your time and interest in the study.

If you are interested in taking part in this study or require any further information then please contact:

Chris Mawhinney **Email:** C.Mawhinney@ljmu.ac.uk

LIVERPOOL JOHN MOORES UNIVERSITY
CONSENT FORM

Project Title:
The effect of cold-water immersion on the thermoregulatory and vascular responses during recovery following exercise

Researcher: Chris Mawhinney

Supervisory Team: Dr Warren Gregson (Director of the project), Dr Helen Jones, Prof. Danny Green, Dr David Low

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential. ☐
4. I agree that any photos taken during the investigations could be used in presentations at national and/or international conferences and/or in journal publications. ☐
5. I agree to take part in the above study. ☐

Name of Participant Date Signature

Name of Researcher Date Signature

Name of Person taking consent Date Signature
(if different from researcher)

APPENDIX 3

Dietary Recording Sheet

Name:

- **Please record your fluid and nutritional intake during the 48 h period prior to your first test.**
- **Please refrain from exercise, alcohol, tobacco and caffeine in the 48-hour period prior to your first test.**
- **On the morning of the exercise trial please refrain from eating until completion of the days testing (overnight fast)**
- **In order to ensure you are completely hydrated prior to testing can you please consume at least 5 ml.kg.bw (_____) of water 2 h prior to arriving at the laboratory. NB. Only consume water on the morning of the exercise trial**

Description of Food/Fluid Intake	Amount (grams/litres)	Time of Intake
e.g. Fresh pasta	300 g	7 pm
e.g. Diluted orange juice	400 ml	7 pm

APPENDIX 4

Influence of Cold-Water Immersion on Limb and Cutaneous Blood Flow after Exercise

CHRIS MAWHINNEY[1], HELEN JONES[1], CHANG HWA JOO[1], DAVID A. LOW[1], DANIEL J. GREEN[1,2], and WARREN GREGSON[1,3]

[1]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UNITED KINGDOM;* [2]*School of Sport Science, Exercise and Health, The University of Western Australia, Perth, AUSTRALIA; and Aspire Academy for Sports Excellence, Doha, QATAR*

ABSTRACT

MAWHINNEY, C., H. JONES, C. H. JOO, D. A. LOW, D. J. GREEN, and W. GREGSON. Influence of Cold-Water Immersion on Limb and Cutaneous Blood Flow after Exercise. *Med. Sci. Sports Exerc.*, Vol. 45, No. 12, pp. 2277–2285, 2013. **Purpose:** This study aimed to determine the influence of cold (8°C) and cool (22°C) water immersion on femoral artery and cutaneous blood flow after exercise. **Methods:** Twelve men completed a continuous cycle exercise protocol at 70% peak oxygen uptake until a core temperature of 38°C was attained. Subjects were then immersed semireclined into 8°C or 22°C water to the iliac crest for 10 min or rested. Rectal and thigh skin temperature, deep and superficial muscle temperature, thigh and calf skin blood flow (laser Doppler flowmetry), and superficial femoral artery blood flow (duplex ultrasound) were measured before and up to 30 min after immersion. Indices of vascular conductance were calculated (flux and blood flow/mean arterial pressure). **Results:** Reductions in rectal temperature were similar (0.6°C–0.7°C) in all three trials ($P = 0.38$). The mean ± SD thigh skin temperature during recovery was 25.4°C ± 3.8°C in the 8°C trial, which was lower than the 28.2°C ± 1.4°C and 33.78°C ± 1.0°C in the 22°C and control trials, respectively ($P < 0.001$). Recovery muscle temperature was also lowest in the 8°C trial ($P < 0.01$). Femoral artery conductance was similar after immersion in both cooling conditions and was lower (~55%) compared with the control condition 30 min after immersion ($P < 0.01$). Similarly, there was greater thigh ($P < 0.01$) and calf ($P < 0.05$) cutaneous vasoconstriction during and after immersion in both cooling conditions relative to the control condition. **Conclusion:** Colder water temperatures may be more effective in the treatment of exercise-induced muscle damage and injury rehabilitation by virtue of greater reductions in muscle temperature and not muscle blood flow. **Key Words:** COOLING, MUSCLE DAMAGE, RECOVERY, DOPPLER ULTRASOUND

BASIC SCIENCES

Localized cold or cryotherapy is frequently applied after acute soft tissue injury in an attempt to minimize the inflammatory response, local edema, swelling, and pain and therefore enhance recovery from soft tissue injury (2). Because inflammation is also integral in the development of exercise-induced muscle damage (27), whole-limb cooling via cold-water immersion is now increasingly applied after exercise to alleviate some of the physiologic and functional deficits associated with exercise-induced muscle damage (2,31).

The physiological effects of cryotherapy are thought to be partly underpinned by reductions in microvascular blood flow to the injured muscle (22,28), which subsequently reduces edema and the induction of inflammatory events (3,8). Whole-limb cold-water immersion is therefore likely to be effective by virtue of its effect on deep muscle blood flow (13,22). We have recently assessed skin and femoral artery blood flow using laser Doppler flowmetry and high-resolution duplex ultrasound, respectively, to provide an indirect estimate of muscle blood flow in the lower limbs in response to cold (8°C) and cool (22°C) water immersion at rest (13). Immersion in both cold and cool water promoted similar reductions in femoral artery blood flow but increased blood flow to the skin in the cold (8°C) water. This suggests that colder water temperatures may induce greater reductions in muscle blood flow at rest and may therefore be more effective in the treatment of exercise-induced muscle damage and injury rehabilitation.

It is well documented that the vascular response to sympathetic stimulation is blunted during exercise and whole-body heat stress compared with rest (11,30,35). Reduced vasoconstrictor responsiveness in the skin when cold (8°C) water immersion is applied after exercise compared with rest may therefore lead to similar changes in skin blood flow to those associated with cool (22°C) water. Such information has important implications for treatments guidelines because the application of cold-water immersion frequently occurs immediately after exercise, when core body and local limb temperatures are elevated. To date, only two studies have

Address for correspondence: Warren Gregson, Ph.D., Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom St Campus, Liverpool, L3 3AF, United Kingdom; E-mail: W.Gregson@ljmu.ac.uk.
Submitted for publication January 2013.
Accepted for publication May 2013.

0195-9131/13/4512-2277/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e31829d8e2e