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**Prolonged Cooling for Exercise
Recovery: A Novel Use for Phase
Change Material**

SY Kwiecien

PhD

2020

**Prolonged Cooling for Exercise
Recovery: A Novel Use for Phase
Change Material**

Susan Yvonne Kwiecien

A thesis submitted in partial fulfilment
of the requirements of Northumbria
University for the degree of Doctor of
Philosophy

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Rehabilitation and in collaboration with
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York, USA

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Abstract

Strenuous exercise can result in structural damage to the skeletal muscle. Muscle damage, which can be experienced by both recreational and elite athletes, is accompanied by signs and symptoms such as strength loss, and increases in soreness, oxidative stress, and inflammation. If excessive or unabated muscle damage can result in performance decrements. Hence, accelerating recovery has been the focus of much research. Cryotherapy has become an increasingly popular recovery modality for its purported ability to reduce blood flow, metabolism and the inflammatory response at the site of the muscle damage. Cold water immersion (CWI) is most commonly used following exercise for reducing muscle soreness, but evidence to support its use for accelerating recovery of strength loss, muscle damage, or inflammation remains conflicting. All cryotherapy modalities are limited in their duration of application. Phase change material (PCM), a novel cryotherapy modality, is capable of overcoming this limitation by extending the duration of treatment for longer than other more traditional cryotherapy modalities. Thus, PCM might present a practical alternative to CWI as a recovery modality. However, the application of PCM for accelerating recovery from exercise has not been examined. Therefore, this course of investigation aimed to elucidate the effects of PCM on recovery from strenuous exercise. Firstly, this research focused on determining the efficacy of prolonged PCM cooling for recovery from mechanically and metabolically stressful exercise. Secondly, the effects of prolonged PCM cooling on intramuscular, core temperature, and the cardiovascular response were determined and compared with a CWI protocol. Finally, this research investigated whether PCM cooling blunted the acute adaptive response to eccentric exercise, following a repeated bout of exercise.

Study 1: This study established the proof of concept that wearing a garment fitted with PCM at a temperature of 15°C administered to the quadriceps for 6 hours could improve recovery from damaging eccentric exercise. The PCM treatment was effective in accelerating recovery of strength loss and soreness. Results also demonstrated that prolonged PCM cooling accelerated recovery of these variables in the leg that did not directly receive PCM cooling. This finding suggested that prolonged PCM cooling might deliver a systemic, and not just a local effect.

Study 2: This investigation examined the efficacy of PCM as a recovery intervention following exercise with a large metabolic component (a marathon run). Unlike the results from Study 1, recovery of strength loss and perceived soreness were not accelerated from 3 hours of PCM cooling. The results also indicated that PCM cooling was not effective in accelerating recovery of vertical jump height, or blood markers of muscle damage or systemic inflammation.

Study 3: This study determined the effects of 3 hours of PCM cooling and 15 minutes of CWI controlled for treatment temperature (15°C PCM and $15 \pm 1^{\circ}\text{C}$ CWI) on intramuscular-, core-, skin-temperature and cardiovascular responses. Although the magnitude of temperature reduction from both PCM and CWI was comparable, PCM maintained a reduction in intramuscular temperature throughout the duration of application. This study also confirmed that, during application, both modalities exerted a central effect on core temperature and heart rate (HR). These effects on core temperature and HR suggested that the effect observed from indirect PCM cooling in Study 1 was a true systemic effect.

Study 4: The final study of this thesis expanded on the findings of the pilot study, by repeating the same exercise protocol 2 weeks later, in order to examine whether acute adaptation (the repeated bout effect; RBE) was influenced by the PCM intervention. In contrast to the first investigation, the PCM were applied to both legs in the treatment condition, and blood markers of muscle damage and inflammation were additionally measured. The results demonstrated that PCM cooling accelerated recovery of strength, soreness, and the blood marker of muscle damage, but not inflammation. Importantly, despite accelerating recovery following the first bout of exercise, PCM did not inhibit the RBE.

The series of investigations encompassing this thesis have established support for the use of prolonged PCM cooling in accelerating recovery from exercise of a mechanical nature. Further, this thesis provides new additions to the literature on the novel application of PCM as a recovery modality for accelerating recovery of strength loss without inhibiting the adaptive response to exercise.

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List of Abbreviations

ANOVA	Analyses of variance
ATP	Adenosine triphosphate
Ca ²⁺	Calcium
CI	Confidence interval
CK	Creatine kinase
CWI	Cold water immersion
DOMS	Delayed onset muscle soreness
E-C coupling	Excitation-contraction coupling
EIMD	Exercise induced muscle damage
ES	Effect size
HR	Heart rate
HRV	Heart rate variability
hsCRP	High sensitivity c-reactive protein
IL-6	Interleukin-6
MIVC	Maximal Isometric Voluntary Contraction
Nm	Newton meters
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
PBC	Partial body cryotherapy
PCM	Phase change material
RBE	Repeated bout effect
RMSSD	Square root of the mean squared differences of successive intervals
ROS	Reactive oxygen species
VAS	Visual analogue scale
VS	Versus
WBC	Whole body cryotherapy

Publications

Peer-Reviewed Publications Arising from this Course of Investigation

Kwiecien, S. Y., O'Hara, D. J., McHugh, M. P., and Howatson, G. (2019) 'Prolonged cooling with phase change material enhances recovery and does not affect the subsequent repeated bout effect following exercise' *European Journal of Applied Physiology*, 120(2), 413-423.

Kwiecien, S. Y., McHugh, M. P., Goodall, S., Hicks, K. M., Hunter, A. M., and Howatson, G. (2019) 'Exploring the Efficacy of a Safe Cryotherapy Alternative: Physiological Temperature Changes from Cold Water Immersion vs Phase Change Material Cooling' *International Journal of Sports Physiology and Performance*, 14(9), 1288-1296.

Kwiecien, S. Y., McHugh, M. P., and Howatson, G. (2018) 'The efficacy of cooling with phase change material for the treatment of exercise-induced muscle damage: pilot study' *Journal of Sports Sciences*, 36(4), 407-413.

Concurrent Related Work - Reviewed Publications during the Course of Investigation

Mullaney, M.J., **Kwiecien, S.Y.,** Fink, A.C., Ioviero, N.M., Howatson, G., and McHugh, M.P. (In-press) 'Accelerated recovery of muscle function in collegiate baseball pitchers using prolonged post-game phase change material cooling.'

Kwiecien, S.Y. (2020) 'Letter to the Editor re 'Volume of water added to crushed ice affects the efficacy of cryotherapy: a randomised, single-blind, crossover trial''. *Physiotherapy*, [Epub ahead of print].

Kwiecien, S.Y., Mathew S., Howatson G., and McHugh, M.P. (2019) 'The effect of varying degrees of compression from elastic vs plastic wrap on quadriceps intramuscular temperature during wetted ice application'. *Scandinavian Journal of Medicine & Science in Sports*, 29(8), 1109-1114.

Clifford, T., Abbott, W., **Kwiecien, S.Y.,** Howatson, G. and McHugh, M.P. (2017) 'Cryotherapy Re-Invented: Application of Phase Change Material for Recovery in Elite Soccer' *International Journal of Sports Physiology and Performance*, 13(5), 584-589.

Conference Communications and Published Abstracts during Course of Investigation

Kwiecien, S. Y., McHugh, M. P., O'Hara, D. J., and Howatson, G. (2019) Phase Change Material Cooling Reduces Indices of Muscle Damage and Does Not Inhibit Acute Adaptation. American College of Sports Medicine Annual Conference. 28 May-1 June 2019. Orlando, Florida.

Kwiecien, S. Y., Mathew, S., McHugh, M. P. and Howatson, G. (2019) The effect of icing with varying degrees of compression on quadriceps intramuscular temperature. American College of Sports Medicine Annual Conference. 28 May-1 June 2019. Orlando, Florida.

Mullaney, M. J., **Kwiecien, S. Y.**, Fink, A., McHugh, M. P. (2019) Accelerated Recovery of Muscle Function in Baseball Pitchers Using Postgame Phase Change Material Cooling: A randomized crossover trial. American Orthopaedic Society for Sports Medicine Annual Conference. 11 July-14 July 2019. Boston, Massachusetts.

Kwiecien, S. Y., McHugh, M. P., Goodall, S., Hicks, K. M., Hunter, A. M. and Howatson, G (2018) 'Effect of Cold Water Immersion versus Phase Change Material Cooling On Core and Intramuscular Temperature'. American College of Sports Medicine Annual Conference. 29 May-2 June 2018. Minneapolis, Minnesota.

Kwiecien, S. Y., McHugh, M. P. and Howatson, G. (2016) – 'The Efficacy of Cooling with Phase Change Material for the Treatment of Exercise Induced Muscle Damage'. American College of Sports Medicine Annual Conference. 1-4 June 2016. Boston, Massachusetts.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work.

Any ethical clearance for the research presented in this thesis has been approved.

Approval was sought and granted by the Faculty of Health and Life Sciences Ethics Committee, or by the Northwell Health Internal Review Board.

I declare that the word count of this thesis is 45, 017 words.

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Date: April 15th, 2020

1.0 INTRODUCTION

Exercise can induce damage to the musculoskeletal, metabolic and nervous systems. Novel, unaccustomed, or exercise performed in excess can result in exercise-induced muscle damage (EIMD). The severity of EIMD is dependent on several factors including the exercise type, duration, intensity and the individual's habituation to the exercise. The deleterious effects associated with EIMD have been well documented (Armstrong, Warren, & Warren, 1991; Ebbeling & Clarkson, 1989; Pyne, 1994). A temporary reduction in muscle force, delayed onset muscle soreness (DOMS), and increased inflammation are all associated with EIMD (Tee, Bosch, & Lambert, 2007). Ultimately decrements in physical performance can result if the signs and symptoms of EIMD are not mitigated appropriately (Burt & Twist, 2011; Twist & Eston, 2008). The human body can recover and adapt from exercise when given a sufficient period of rest. However, due to limited windows between sessions during training and competition phases, full recovery does not occur in most athletes. A chronic imbalance between training stress and recovery can ultimately lead to underperformance as well as overtraining and burnout (Barnett, 2006; Minett & Duffield, 2014). Thus, accelerating recovery from exercise, in order to return the body to its pre-exercise state and maximise subsequent performance, has been the focus of much research.

Recovery interventions are touted to decrease the signs and symptoms of EIMD. Athletes have turned to an abundance of recovery modalities, such as antioxidant supplements, nonsteroidal anti-inflammatory drugs, and cryotherapy, in an attempt to improve recovery following exercise (Bishop, Jones, & Woods, 2008; Kovacs & Baker, 2014; Nédélec et al., 2012; Robson-Ansley, Gleeson, & Ansley, 2009). Cold water immersion (CWI), in temperatures ranging between 10°C and 15°C for durations of 12.5 ± 5.6 minutes on average (Bleakley et al., 2010), is one of the most popular recovery strategies used following exercise (Leeder, Gissane, van Someren, Gregson, & Howatson, 2012). Its popularity has been advocated by research implementing its use following isolated eccentric exercise (Machado et al., 2016; Vaile, Halson, Gill, & Dawson, 2007), endurance exercise (Brophy-Williams, Landers, & Wallman, 2011; Yeargin et al., 2006), resistance exercise (Roberts, Muthalib, et al., 2015), laboratory cycling protocols (Halson et al., 2008; Lane & Wenger, 2004; Peiffer, Abbiss, Watson, Nosaka, & Laursen, 2010; Vaile et al., 2010; Vaile, Halson, Gill, & Dawson, 2008) and team-sport exercise (Garcia, da Mota, & Marocolo, 2016; Leeder et al., 2019; Montgomery et al., 2008; Rowsell, Coutts, Reaburn, & Hill-Haas, 2009, 2011; Webb, Harris, Cronin, & Walker, 2013). Recent meta-analyses have

demonstrated CWI to be efficacious in alleviating DOMS for up to 96 hours post-exercise (Bleakley et al., 2010; Leeder et al., 2012; Versey, Halson, & Dawson, 2013).

Although CWI has received much attention in recent literature, there remains variability and disparity in the evidence supporting its use for accelerating the overall recovery process, and no consensus exists for optimal treatment criterion. This is, in part, due to large variations in temperature and duration of immersion of CWI protocols. The underlying mechanisms through which CWI might accelerate recovery remain to be elucidated. However, CWI is purported to aid recovery from EIMD through a decrease in muscle temperature (Ihsan, Watson, & Abbiss, 2016; White & Wells, 2013; Wilcock, Cronin, & Hing, 2006). It has been suggested that a decrease in muscle temperature and local circulation reduces cellular metabolism, which consequently limits secondary damage (Merrick, Rankin, Andres, & Hinman, 1999). Unfortunately, these effects have only been directly demonstrated in animal models and remain to be replicated in humans. Investigators have suggested that a more aggressive regimen increasing duration, and alternating frequency or temperature of the immersions, could be employed with better results (Goodall & Howatson, 2008).

The duration of cryotherapy can be prolonged by utilising a cooling medium called phase change material (PCM). While changing phase from a solid to a liquid, PCM absorbs and stores heat while maintaining a constant temperature for the entirety of melting. This is known as the latent phase, which correlates with the melting point of the PCM. The most commonly utilised PCM is ice, which experiences its latent phase at 0°C. Since the latent phase of ice occurs at a relatively low temperature, the duration of melting is relatively short. Fortunately, the temperature of the latent phase of any PCM can be manipulated. As a result, PCM experiencing its latent phase at a temperature greater than 0°C will maintain the latent phase for a longer duration than ice, for example. Most research to date has focused on administering PCM, with latent phases between 10 to 31°C, in a cooling vest to improve thermal comfort (Barwood, Davey, House, & Tipton, 2009; Brade, Dawson, Wallman, & Polglaze, 2010; Purvis & Cable, 2000; Tate, Forster, & Mainwaring, 2008). These studies demonstrated that PCM with a latent phase of 24°C had a stronger cooling effect on torso skin temperature than that with a latent phase of 28°C (Gao, Kuklane, & Holmér, 2011). The longest treatment duration in any of these studies was 90 minutes with no adverse or negative effects of the skin (Gao, Kuklane, Wang, & Holmér, 2012). Consequently, PCM with a latent phase between 10-15°C was suggested to be most effective for body cooling (Gao et al., 2011; House et al., 2013). To date, only one study has examined the effects of PCM as a recovery method on physiological and

thermoregulatory responses (Hauswirth et al., 2012), demonstrating that PCM exerts greater benefits on physiological load compared to CWI in the early stage of subsequent exercise.

Wearing a garment fitted with PCM might be more effective and logistically simpler to implement than CWI. Given the widespread use and acceptance by athletes of CWI as a recovery modality, with limited empirical evidence supporting its use for overall functional recovery, it remains important to explore alternative solutions. Further, the use of PCM offers a substitute to CWI as it might allow for specific manipulation in the frequency, duration, and temperature of treatment. In particular, utilising PCM with a phase point of 15°C as an alternative to other cryotherapy modalities would provide a solution to prolonging the duration treatment. To date, no literature exists utilising PCM to enhance recovery following training and competition in elite athletes or recreational populations. The efficacy of PCM cooling for recovery from EIMD has not been investigated. Hence, the overarching aim of this thesis was to test the efficacy of prolonged cooling using PCM to facilitate accelerated recovery from exercise.

Specifically, this aim was addressed in 4 experimental chapters:

- 1) To establish the efficacy of a novel cooling strategy (PCM) as an intervention on indices of strength and soreness from an exercise protocol involving high mechanical stress, designed to induce damage.
- 2) To establish the efficacy of PCM as a recovery intervention on indices of strength, soreness, functional performance, and blood markers of muscle damage and inflammation following exercise involving high metabolic stress, a marathon run.
- 3) To compare the physiological effects (skin, muscle, core temperature and cardiovascular measures) of PCM cooling with a temperature matched CWI protocol.
- 4) i. Determine whether prolonged PCM cooling accelerates recovery from exercise involving mechanical stress, in part by inhibiting the inflammatory effect compared to a control.
ii. Examine whether the accelerated recovery from an initial bout of strenuous quadriceps exercise would affect the response to a subsequent bout of strenuous quadriceps exercise.

2.0 LITERATURE REVIEW

This literature review is divided into two parts. Part 2.1 discusses the physiological response to exercise, with particular focus placed upon muscle damage and inflammation. Part 2.2, firstly examines the use of cryotherapy for recovery of the exercise-induced responses presented in part 2.1, with a specific focus on cold water immersion (CWI). Secondly, the use of a novel cryotherapy recovery strategy, phase change material (PCM), is introduced as a supplement to the review of cryotherapy literature. Detailed analysis of certain related issues, such as the hemodynamic response to exercise, the use of cryotherapy for management of acute soft tissue sports injury, the use of cryotherapy for thermoregulation during and from exercise performed in the heat, or an in-depth discussion of the molecular inflammatory cascade triggered by exercise and the molecular response to cryotherapy (Broatch, Petersen, & Bishop, 2018) as well as the influence of cryotherapy on the adaptive response to exercise, are beyond the scope of this review.

2.1 Physiological Response to Exercise

The processes that transpire within the active muscle during exercise are initiated rapidly and occur in several stages (Armstrong et al., 1991; Armstrong, 1990; Järvinen, Järvinen, Kääriäinen, Kalimo, & Järvinen, 2005; Lieber, 2018). These events can be divided into the initial damage response, followed by a secondary damage response, and culminating in a regenerative/repair phase. The initial phase encompasses structural damage of the muscle fibre, followed by impaired excitation-contraction (E-C) coupling and calcium ion (Ca^{2+}) signalling, and activation of degradation pathways (Peake, Nosaka, & Suzuki, 2005). The subsequent secondary stage involves a proliferation of the initial muscle injury, coupled with an inflammatory response surrounding the damaged muscle. Following the progression of catabolic events, the muscle fibre returns to its pre-exercise condition, and cellular homeostasis and physiological function are restored. The time course of this cycle is dependent on the magnitude of muscle damage and is understood to take several days (Paulsen, Mikkelsen, Raastad, & Peake, 2012). The sequence of physiological processes occurring as a result of exercise will be detailed in the following sections.

2.1.1 The Stress Response to Exercise Stimulus

Three pathways exist that result in exercise-induced structural damage to the muscle (Figure 1). In the first pathway, mechanical stress to the muscle directly results from unaccustomed exercise of eccentric nature (Fridén & Lieber, 2001; Proske & Morgan, 2001) involving accelerations/decelerations (Akenhead, Hayes, Thompson, &

French, 2013) and/or changes in direction. This type of stress can involve downhill running or sports involving cutting motions such as American football, field hockey, or rugby. Eccentric muscle actions involve force generation in a lengthening muscle (Stauber, 1989) and are associated with a higher force-to-activation ratio (Lieber, 2018), recruitment of fewer total muscle fibres (Enoka, 1996), and an overall greater recruitment of type II muscle fibres which are more susceptible to damage compared to type I fibres (Fridén, Sjöström, & Ekblom, 1983; Magal et al., 2010). As a result, the net mechanical stress per muscle fibre is increased (Enoka, 1996), and structural damage occurs directly due to a failure in the myofibrils during eccentric contractions.

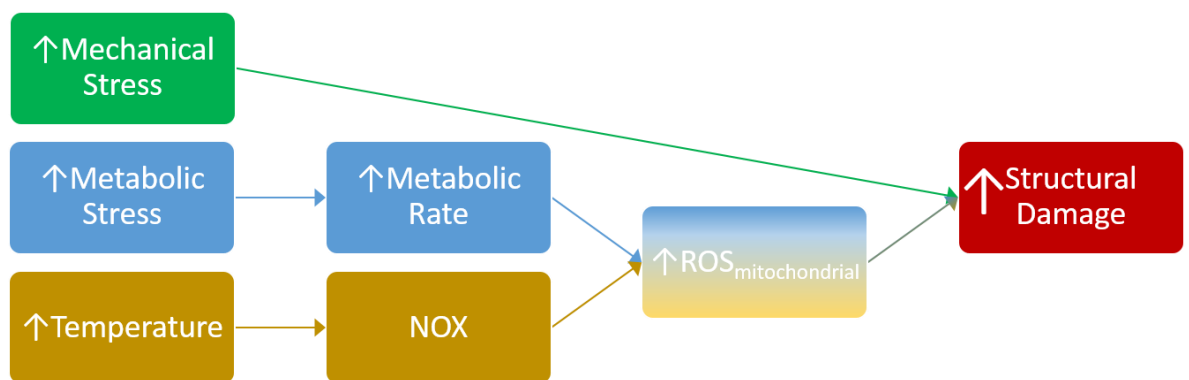


Figure 1: Three pathways to exercise-induced structural damage. Mechanical stress causes structural damage to the muscle fibre directly. On the other hand, metabolic stress and the increase in temperature during exercise cause structural damage indirectly through an increase in the metabolic rate and nicotinamide adenine dinucleotide phosphate oxidase (NOX) activity, respectively. Both result in an increase in mitochondrial reactive oxygen species (ROS) activity.

In the second pathway, exercise of prolonged duration and/or high metabolic intensity such as marathon running results in metabolic stress. In the metabolic damage model, the initial events resulting in EIMD are caused by metabolic deficiencies (Tee et al., 2007). Metabolic stress occurs when the rate of adenosine triphosphate (ATP) hydrolysis exceeds that of ATP synthesis (Baird, Graham, Baker, & Bickerstaff, 2012), due to dysfunction of the Ca^{2+} -ATPase pumps in the sarcoplasmic reticulum or sarcolemma, resulting in a reduction in the concentrations of high energy phosphates and an increase in cytosolic Ca^{2+} (see section 2.1.2). Depletion of ATP increases the rate of aerobic energy transformation (Clanton, 2007) and the metabolic rate at the site of the mitochondria. This triggers an increase in mitochondrial reactive oxygen species (ROS; Spiteller, 2006; Supinski & Callahan, 2007), denaturing of

proteins, nucleic acids and lipids. The result is the aforementioned structural damage that occurs as a direct result of mechanical damage, and concomitantly a change in the redox status of the cell, which alters the function of redox-sensitive transcription factors. Some of these transcription factors are involved in muscle adaptation, such as increasing the antioxidant status and stimulating mitochondrial biogenesis and enzymes, while others are involved in the production and secretion of cytokines. These metabolic deficiencies ultimately increase the vulnerability of the muscle fibre to mechanical stress.

The third pathway that results in exercise-induced structural damage to the muscle occurs independently from the mechanical and/or metabolic stress responses. Exercise induces heat generation (Arbogast & Reid, 2004), which increases the concentration of nicotinamide adenine dinucleotide phosphate oxidase (NOX) within the muscle fibre resulting in an increase in the production of ROS from the mitochondria and from the infiltrating inflammatory cells (Powers & Jackson, 2008). Concomitantly these effects contribute to the structural damage in the muscle as well as to the disruption in the homeostasis of other physiological systems within the body (White & Wells, 2013). Importantly, the exercise stimulus can be a combination of both metabolic and mechanical stresses. Most team sports, such as football, encompass both a metabolic component, due to the prolonged duration of the exercise stimulus, and a mechanical component, due to the cutting motions made on the field.

2.1.2 Exercise-Induced Muscle Damage (EIMD)

Following muscle damage two phases, the initial phase and a delayed secondary phase, have been described to transpire (Faulkner, Brooks, & Opitck, 1993). The initial stress response to exercise results in impairment of normal skeletal muscle cell function called exercise-induced muscle damage (EIMD; Clarkson, 1992; Clarkson & Tremblay, 1988; Clarkson & Hubal, 2002; Fridén & Lieber, 1992). During this primary phase of damage, the rapid activation of processes mentioned in the previous section (2.1.1) results in structural damage to the skeletal muscle (for review see: Clarkson & Sayers, 1999; Proske & Allen, 2005). Specifically, the initial damage response involves both disrupted sarcomeres and damage to the E-C coupling system (Proske & Morgan, 2001). Compromised structural integrity at the level of Z line, A band, and the contractile system leads to sarcomere disruption in the myofibrils (Armstrong et al., 1991; Fridén & Lieber, 1992; Lieber, Thornell, & Fridén, 1996; Nosaka & Clarkson, 1995; Proske & Morgan, 2001). While a failure in the sequence of events that starts

with the release of acetylcholine at the neuromuscular junction and ends with the release of Ca^{2+} from the sarcoplasmic reticulum leads to E-C uncoupling (Warren, Ingalls, Lowe, & Armstrong, 2001).

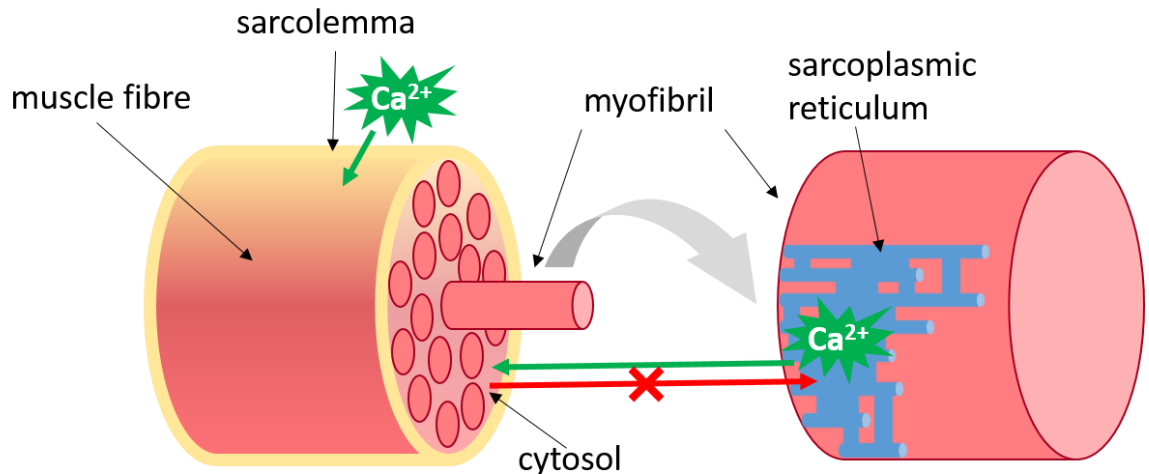


Figure 2: Calcium (Ca^{2+}) activity during the primary phase of exercise-induced muscle damage. Structural damage at the level of the muscle fibre makes the sarcolemma more permeable to Ca^{2+} , which can then enter the cytosol from external sources. Simultaneously, the sarcoplasmic reticulum, which typically stores Ca^{2+} ions, excretes Ca^{2+} into the cytosol instead of absorbing it. The result is a loss of Ca^{2+} homeostasis.

Ultimately, structural damage of the muscle is induced by a cascade of events characterised by a loss of Ca^{2+} homeostasis (Armstrong et al., 1991; Armstrong, 1990; Fatouros & Jamurtas, 2016). Destabilisation of the sarcolemma due to both Na-K-ATPase and Ca^{2+} -ATPase pump dysfunction (Baird et al., 2012) and subsequently uncoupling of the E-C coupling system (Ingalls, Warren, Williams, Ward, & Armstrong, 1998b; Warren, Ingalls, Lowe, & Armstrong, 2001), makes the muscle fibres more permeable to Ca^{2+} entry (Figure 2; Cho, Woo, Perez, & Lee, 2017). Simultaneously, sustained release of Ca^{2+} from the sarcoplasmic reticulum, which typically stores Ca^{2+} ions, increases cytosolic Ca^{2+} concentration within the muscle fibre, which activates intracellular proteolytic enzyme activity and signalling of inflammatory cells (Belcastro, Shewchuk, & Raj, 1998; Fatouros & Jamurtas, 2016; McArdle & Jackson, 1997; Peake et al., 2005), increasing intramuscular pressure. The result is fragmentation of the sarcoplasmic reticulum which leads to: impaired

Ca²⁺ sequestration (Byrd, 1992), damage to the extracellular matrix and cytoskeleton, and/or swollen mitochondria (Armstrong, 1990; Fridén & Lieber, 1992).

Elevations in intracellular Ca²⁺ activate several proteolytic and phospholipolytic pathways, which respectively degrade structural and contractile proteins and membrane phospholipids (Armstrong et al., 1991). Simultaneously, muscle cell permeability is reduced while capillary permeability and osmolality are increased, resulting in the leakage of these proteins and membrane phospholipids and disruption of intracellular homeostasis. For example, the disruption in homeostasis and permeability of the sarcolemma enables the diffusion of soluble muscle enzymes, such as serum creatine kinase (CK), to leak from the muscle into the interstitial fluid and extracellular space, ultimately reaching the circulation (Armstrong et al., 1991; Baird et al., 2012; Clarkson, Byrnes, Gillis, & Harper, 1987b; Magal et al., 2010; McLellan, Lovell, & Gass, 2010). Other intramuscular proteins such as myoglobin (Lindsay, Carr, Draper, & Giese, 2015), metabolites (Ebbeling & Clarkson, 1989), cytokines (Robson-Ansley, Milander, Collins, & Noakes, 2004; Vargas & Marino, 2014), heat shock proteins (demonstrated in animal models; Ingalls, Warren, & Armstrong, 1998a), and immune function markers such as C-reactive protein (CRP; Cunniffe et al., 2010) are also released. Following exercise, these are routinely measured in the circulation to infer the level of homeostatic perturbation indirectly associated with EIMD.

Direct signs such as histological evidence (e.g. muscle-tendon shortening, loss of muscle contractile function) during the initial period of disruption of the myofibrillar structure (Fridén & Lieber, 1992), and indirect signs such as swelling, inflammation (Smith, 1991), and oxidative stress (see Fatouros & Jamurtas, 2016 for review) can be used to characterise the severity of EIMD. Symptoms following EIMD such as increased passive stiffness, localised soreness, and strength loss (Clarkson & Tremblay, 1988; Clarkson & Newham, 1995; Clarkson & Hubal, 2002; Cleak & Eston, 1992; Eston, Finney, Baker, & Baltzopoulos, 1996; Fridén & Lieber, 2001; Hellebrandt, Houtz, Hockman, & Partridge, 1956; Nicol, Avela, & Komi, 2006; Proske & Morgan, 2001; Talag, 1973; Warren, Lowe, & Armstrong, 1999; White, Rhind, & Wells, 2014; White & Wells, 2013), can also be used to quantify the extent of the muscle damage.

Prolonged strength loss following damaging exercise is considered to be one of the most valid and reliable indirect measures of EIMD in humans (Clarkson & Hubal, 2002; Warren et al., 1999). Following exercise the reduction in strength has been

related to an inactivation of the cation pumps within the muscle. In particular, fatigue induced by prolonged exercise results in a decline in Na-K-ATPase activity; which is most depleted at the cessation of exercise (Leppik et al., 2004). Concomitantly, sarcoplasmic reticulum Ca²⁺-ATPase function has been shown to remain depressed for up to 36-48 hours post-exercise (Tupling, Green, Roy, Grant, & Ouyang, 2003). Delayed onset muscle soreness (DOMS), the delayed discomfort incurred from the EIMD process (Armstrong, 1984; Byrnes et al., 1985; Cheung, Hume, & Maxwell, 2003; Gulick & Kimura, 1996; Schutte & Lambert, 2001), is the most commonly reported symptom of EIMD. However, DOMS might not accurately reflect the magnitude of EIMD and inflammation, as changes in indirect markers of muscle damage and inflammation are not necessarily accompanied by DOMS (Nosaka, Newton, & Sacco, 2002). Hence, it is imperative to mention that DOMS may not necessarily correlate with changes in muscle function (Newham et al., 1983; Newham et al., 1987; Clarkson & Ebbeling, 1988; Warren et al., 1999).

Currently, there is no 'gold standard' for measuring EIMD. Performing histological observations or measuring changes in force-generating capacity seem to be the most valid markers of EIMD; however, both have limitations. Muscle biopsy samples might not be representative of the whole muscle, and acute muscle damage may occur as a result of the biopsy procedure itself. On the other hand, following exercise, the muscle can experience a decline in the amount of force or power capacity that a muscle can typically exert (Enoka, 2008). Thus, the change in a muscle's force-generating capacity, best measured as a maximal isometric voluntary contraction (MIVC), provides a good indication of the status of the whole muscle (Warren et al., 1999) and can be used to indirectly quantify EIMD. However, MIVC is often measured in isolated muscle groups. Therefore, MIVC likely does not reflect the complete picture of muscle function associated with activities involving multiple muscle groups such as marathon running or a football match (Byrne, Twist, & Eston, 2004). Hence, countermovement jump (CMJ) performance, which reflects impairments in stretch-shortening cycle function (Komi, 2000), may be used concurrently with MIVC to provide a more complete picture of dynamic muscle function. Ultimately, tests of muscle function are useful because they span the whole period of muscle degeneration and regeneration that occurs following EIMD (Warren et al., 1999).

A combination of methods, including other indirect markers of muscle damage, such as DOMS and circulating serum CK, might be the most complete way to quantify the magnitude of EIMD. Serum CK is the most commonly measured protein in EIMD studies because it is a reliable indicator of muscle membrane permeability found

exclusively in muscle tissue (Pyne, 1994). The appearance of CK in blood has been generally considered to be an indirect marker of muscle damage (Baird et al., 2012). There is controversy in the literature concerning the validity of CK in reflecting muscle damage because the magnitude of eccentric contractions involved in the exercise and the subsequent extent of muscle disruption both influence serum CK response (Baird et al., 2012; Magal et al., 2010). Further, there is a clear discrepancy between the time of peak serum CK levels and peak muscle soreness (Clarkson, Apple, Byrnes, McCormick, & Triffletti, 1987a; Clarkson & Ebbeling, 1988; Newham, Jones, & Edwards, 1983). Thus, CK values should be utilised concomitantly with other blood markers when elucidating the degree of muscle damage.

2.1.2.1 Secondary Damage to Skeletal Muscle

The initial structural damage occurring within the muscle fibre initiates a positive feedback mechanism during which the aforementioned initial damage response is exacerbated (Kendall & Eston, 2002; Lapointe, Frémont, & Côté, 2002a). A cascade of events is triggered by the initial loss of Ca^{2+} homeostasis, involving disruption in intracellular homeostasis followed by activation of neutrophils resulting in an inflammatory response (Howatson & van Someren, 2008; Toumi & Best, 2003; see section 2.1.2.2). This phase of muscle damage is referred to as the secondary damage response. Due to the increased permeability at the site of damage, the concentration of intracellular Ca^{2+} from extracellular sources is increased (Armstrong et al., 1991), further exacerbating the loss of Ca^{2+} homeostasis within the muscle fibre (Duncan, 1987; Gissel & Clausen, 2001). Other events compounding the overall structural damage include: endothelial and intramuscular degradation (Howatson & van Someren, 2008), initiated by protease activity which degrades cytoskeletal proteins within the muscle (Clarkson & Sayers, 1999); an increase in the destructive activity of lysosomal enzymes surrounding the primary damaged tissue (Merrick, 2002); decreased nutritive blood flow, reduced oxygen delivery, and elevated cellular metabolism at the site of structural damage (Harris et al., 1986; Hill & Hill, 1998; Schaser et al., 1999; Zhang, Bail, Mittlmeier, Haas, & Schaser, 2003); and leukocyte invasion at the site of the muscle fibre cell wall (Paulsen et al., 2012) which results in potentiation of the inflammatory response. Ultimately, secondary muscle damage compounds the symptoms of EIMD and results in accumulation of metabolites, increased intramuscular pressure (Ebbeling & Clarkson, 1989) and hypoxic stress on the muscle (Andersson, Karlsen, Blomhoff, Raastad, & Kadi, 2010; Armstrong, 1984; Ascensão et al., 2008), leading to impaired muscle function in the hours and days following exercise (Lapointe et al., 2002a; Merrick et al., 1999). If not managed

correctly, these effects can be detrimental to an athlete's recovery and subsequent performance.

2.1.2.2 Muscle Inflammation from Exercise

Although inflammation can be induced through mechanical, metabolic, chemical, thermal, or microbial stimuli (Hurley, 1983), this section will focus on the inflammatory response to EIMD (for thorough review see Clarkson & Sayers, 1999; Kendall & Eston, 2002; McArdle & Jackson, 1997; Smith, 1991; Tee et al., 2007; Tidball, 1995). Importantly, the underlying aetiology of the inflammatory response following mechanical or metabolic stress (described in section 2.1.1) is different (Figure 3; Tee et al., 2007; Toumi & Best, 2003; White & Wells, 2013). Briefly, exercise induces an increase in the production of ROS by neutrophils, either directly at the site of structural damage from mechanical stress or indirectly through the increase in mitochondrial ROS from metabolic stress (Figure 1). The result is an increase in the inflammatory stress signal resulting in an acute inflammatory response to occur (MacIntyre, Reid, Lyster, Szasz, & McKenzie, 1996).

Muscle fibres contain proteolytic enzymes which initiate the degradation of lipid and protein structures following structural damage (Belcastro et al., 1998; Proske & Allen, 2005; Tidball, 2005). The inflammatory response stimulates leukocyte emigration (neutrophil and macrophage invasion) through the endothelial lining to the damaged area (Abrams, 1997; Paulsen et al., 2010), which subsequently influence the trafficking of inflammatory cells (Adams et al., 2011; Fatouros & Jamurtas, 2016; Peake et al., 2005). This process initiates a positive feedback mechanism characteristic of the inflammatory response. In this feedback loop leukocytes are attracted to the cell to break down and clear away damaged tissue, but might also exacerbate the ROS response at the muscle (see Paulsen et al., 2012 for review) further contributing to the structural damage and propagating the positive feedback pattern of the inflammatory response (Butterfield, Best, & Merrick, 2006). Importantly, the direct release of mitochondrial ROS during the metabolic stress pathway additionally triggers the inflammatory stress signal through the change in redox status of the muscle cell which stimulates the release of cytokines (Brown, Day, & Donnelly, 1999).

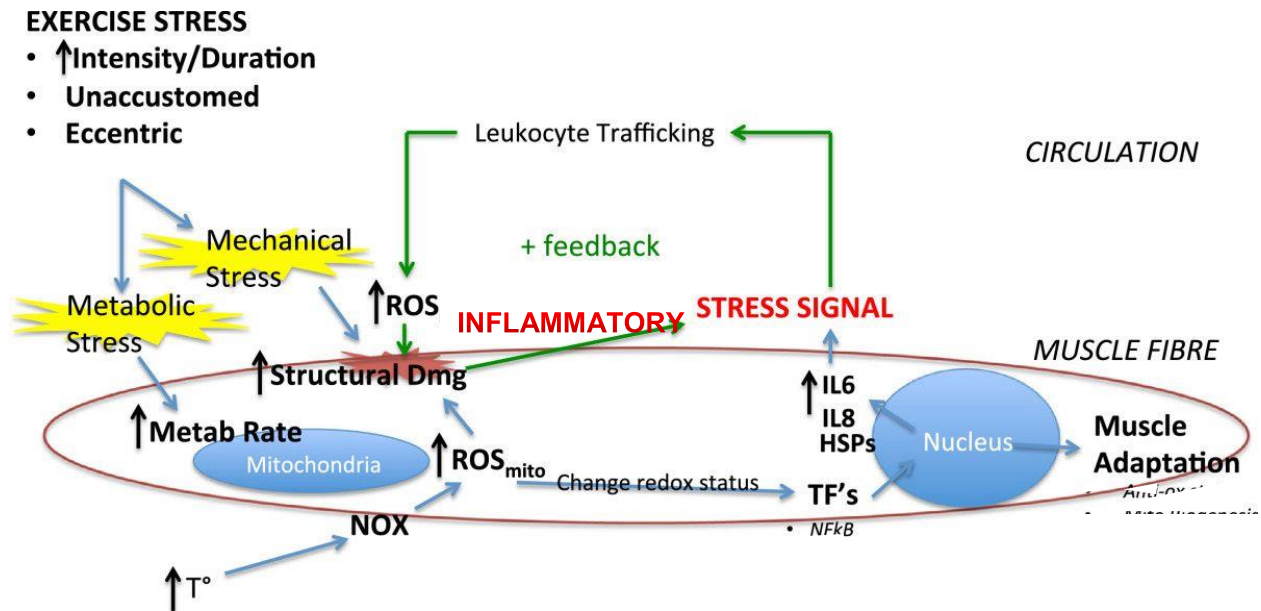


Figure 3: Exercise-induced muscle fibre cell signalling resulting in structural damage (adapted from White & Wells, 2013); T°: temperature; NOX: nicotinamide adenine dinucleotide phosphate oxidase; ROS: reactive oxygen species; IL-6, -8: interleukin-6, -8; HSPs: heat shock proteins; TF's: transcription factors.

The inflammatory processes are important in the removal of injured tissue and for stimulating regeneration of the damaged fibres (Armstrong et al., 1991; Paulsen et al., 2012). Following exercise, the inflammatory process ultimately results in the structural and functional repair of damaged tissues (Tidball, 2005). Inflammation can also proliferate hypertrophic muscle adaptations (Schoenfeld, 2012) and the strengthening of muscle structural elements (Lapointe et al., 2002a). Thus, inflammation is necessary for the adaptive response to exercise (Powers & Jackson, 2008; Tidball & Villalta, 2010). On the other hand, an exacerbated inflammatory response can delay muscle regeneration and contribute to secondary muscle damage (Brickson et al., 2003; Butterfield et al., 2006; Merrick, 2002; Pizza, Koh, McGregor, & Brooks, 2002). Importantly, the overall magnitude of the inflammatory response is dependent on the extent of myofibrillar damage, which regulates the degree of leucocyte accumulation (Paulsen et al., 2012). For example, eccentric exercise, which elicits the greatest extent of structural tissue damage, evokes a greater inflammatory response than concentric exercise. The interactions between muscle and inflammatory cells as well as the history of previous muscle use also play a role in determining the severity of the inflammatory response (Fridén et al., 1983; Fridén & Lieber, 2001; Magal et al., 2010; Paulsen et al., 2012; Tidball, 1995).

2.1.3 Repeated-Bout Effect (RBE)

Exercise disrupts homeostasis and induces an adaptive response, dependent on the type, duration, and intensity of the exercise and training status of the individual. An adaptation called the repeated bout effect (RBE; Clarkson et al., 1987b; Clarkson, 1992; Clarkson & Tremblay, 1988) occurs following a single bout of unaccustomed exercise. However, the magnitude of the RBE is independent of the initial exercise volume (Howatson et al., 2007). Various theories exist to explain the mechanisms involved in the RBE (Howatson, Someren, & Hortobágyi, 2007; Hyldahl et al., 2017; McHugh, 2003; McHugh, Connolly, Eston, Gattman, & Gleim, 2001; McHugh, Connolly, Eston, & Gleim, 1999). The adaptive response manifests as an attenuation in the physiological response to subsequent exercise (Evans et al., 1986), such as influx of CK concentration, myoglobin, symptoms of soreness and decrements in performance (Brown, Child, Day, & Donnelly, 1997; Ebbeling & Clarkson, 1989; Howatson, Someren, & Hortobágyi, 2007; McHugh, Connolly, Eston, & Gleim, 1999; McHugh, Connolly, Eston, Gattman, & Gleim, 2001; Nosaka & Clarkson, 1995; Nosaka & Newton, 2002; Nosaka, Sakamoto, Newton, & Sacco, 2001). This protective effect is evident from upper limb exercise separated by 1 week (Clarkson, Litchfield, Graves, Kirwan, & Byrnes, 1985), after bouts of downhill running separated by 6, but not 9, weeks (Byrnes et al., 1985; Jones et al., 1986), and has been observed following endurance running, cycling, weightlifting, isometric exercise, downhill running and eccentric exercise). Although the duration of the protective effect differs between studies, it seems to be greatest within 2 weeks of the preceding bout and diminishes over time (Nosaka et al., 2001).

2.1.4 Summary

Athletes often experience novel exercise, or exercise of prolonged duration, and are regularly exposed to multiple exercise sessions in one day, or repeat exercise sessions in a tournament scenario or a season of sport. Such exposures commonly cause some degree of EIMD. Sufficient rest will return the cascade of events resulting in EIMD to baseline. However, in many circumstances, athletes only experience partial recovery due to the performance demand of athletic seasons (Halson et al., 2002; Montgomery et al., 2008). Thus, when left unabated in athletes, the signs and symptoms of EIMD result in reductions in both performance and subsequent training (Khan et al., 2016); and the impairments associated with the primary and secondary damage responses are often exacerbated. Although the RBE offers an adaptive response to exercise and protection against both primary and secondary muscle

damage, muscle damage can be induced in trained individuals, even if the stages of muscle damage are shortened and the overall magnitude of EIMD is less in trained athletes than in untrained individuals.

2.2 Cryotherapy for Recovery Following Exercise

Rapid deployment of a treatment strategy is important for athletes to recover from the EIMD described in part 2.1 of this literature review. For this reason, several nutritional, pharmaceutical, and physical recovery strategies are currently popular among athletes (Howatson & Van Someren, 2008; Peake, 2019). Some of these recovery strategies include antioxidant consumption such as tart cherries, nonsteroidal anti-inflammatories, therapeutic ultrasound, transcutaneous electrical nerve stimulation, compression, manual therapy techniques such as massage, and even sleep. These techniques are beyond the scope of this review. Part 2.2 investigates the literature surrounding the use of cryotherapy for recovery following exercise, paying particular attention to the CWI literature. The most thoroughly investigated and most popular cryotherapy modality utilised by athletes for recovery is CWI. The efficacy of cryotherapy on repeat performances within a short recovery period (same day) will not be discussed, as it is contraindicated and the majority of research has demonstrated negligible and detrimental effects from this practice. The shortcomings of CWI for recovery from exercise provide a rationale for the focus of this thesis, which involves prolonging the duration of cooling using PCM as a recovery intervention.

2.2.1 Introduction to Cryotherapy

Cryotherapy, the reduction of tissue temperature by the withdrawal of heat from the body (Michlovitz, 1990), has been utilised as a medical intervention dating back to ancient civilisations. The most common application for cryotherapy at present, in the form of ice, is in the management of or rehabilitation from acute soft tissue injury such as strains, sprains, contusions, as well as fractures (Bleakley, McDonough, & MacAuley, 2004; Knight, 1985; Knight, 1995; Knight, Brucker, Stoneman, & Rubley, 2000; Swenson, Swärd, & Karlsson, 1996). In the treatment of musculoskeletal trauma, the goal for acute management with cryotherapy is to minimise the risk of further damage to the musculature by preventing the proliferation of secondary damage (Knight, 1995; Knight et al., 2000; Merrick, 2002; Merrick & McBrier, 2010). Since cryotherapy can reduce the severity of the initial inflammatory response and promote recovery following traumatic injury, it might also facilitate recovery of muscle tissue experiencing damage and inflammatory changes associated with exercise

(Eston & Peters, 1999; Meeusen & Lievens, 1986; Merrick et al., 1999). For this reason, the application of cryotherapy has been adopted as a popular recovery modality aimed at limiting and accelerating recovery from exercise-induced decrements in performance (Versey et al., 2013). The mechanisms of cooling as they pertain to recovery from exercise are reviewed in subsequent sections.

2.2.2 Cryotherapy in Animal Models

Animal models have shown that a window of opportunity for intervention with cryotherapy lies within the first 30 minutes after injury (Merrick & McBrier, 2010), indicating that cryotherapy interventions should be administered before secondary damage begins to intensify the signs and symptoms of EIMD. Importantly, animal models have demonstrated that cellular metabolic activity (Osterman et al., 1984; Sapega et al., 1988) and oxygen demand (Fuhrman, Fuhrman, Farr, & Fail, 1961; Fuhrman, 1959) are reduced when muscle temperature is decreased to 10°C-15°C. Hence, the rationale for administering cryotherapy treatment following EIMD is that a reduction in metabolic rate will suppress the secondary damage response (Merrick et al., 1999). Cryotherapy treatment in animal models delivering lesser degrees of intramuscular cooling have also demonstrated suppressed metabolic activity (Puntel et al., 2012), as well as a reduced inflammatory response (Amon, Menger, & Vollmar, 2003; Lee et al., 2005; Puntel et al., 2011; Schaser et al., 2006; Vieira Ramos et al., 2016), oedema formation (Deal, Tipton, Rosencrance, Curl, & Smith, 2002; Dolan, Thornton, Fish, & Mendel, 1997; Lee et al., 2005; Schaser et al., 2006; Smith et al., 1993), and tissue damage (Bleakley & Hopkins, 2010; Merrick et al., 1999; Puntel et al., 2012; Schaser et al., 2007).

Animal models have demonstrated conflicting evidence on the effect of repeat cryotherapy exposure on collagen formation. The remodelling phase of muscle regeneration involves the deposition of collagen, necessary for the regeneration of new myofibres. An excessive collagen response leads to excessive fibrosis. One study revealed repeat cryotherapy exposure over 4 weeks led to fibrosis due to excessive collagen deposition (Takagi et al., 2011), while one study applying repeat cryotherapy treatments over 2 weeks did not (Vieira Ramos et al., 2016). This discrepancy could be a result of the different means through which injury was induced, especially since the crush injury method as performed by Takagi et al. (2011) was likely more destructive than a localised 'freeze injury' (Vieira Ramos et al., 2016). To date, EIMD has not been induced directly in animal models. As a result, animal models involving blunt trauma (Curl et al., 1997; Deal et al., 2002; Dolan et al., 1997;

Lee et al., 2005; Puntel et al., 2011; Schaser et al., 2006; Smith et al., 1993), ischemia injury (Puntel et al., 2012) or chemically induced muscle damage (Amon et al., 2003) result in severe damage responses that are not comparable to human injury or EIMD models.

Importantly, rodent muscle has different metabolic properties and responses to environmental stimuli than human skeletal muscle (Kowalski & Bruce, 2014). Thus, changes in muscle temperature and blood flow induced by cryotherapy might be more extensive in animals compared with human muscles. Additionally, the extent of cooling in some of the animal studies demonstrating beneficial results for recovery is of longer duration than what is typically used clinically in humans (3-6 hours; Merrick et al., 1999; Puntel et al., 2012; Sapega et al., 1988; Schaser et al., 2007). For these reasons, direct translation to the clinical setting in humans is limited (Bleakley et al., 2011), and findings from animal studies cannot be directly applied to form the basis of cryotherapy application for exercise recovery in humans.

2.2.3 Effect of Cryotherapy on Body Temperature

The effect of tissue cooling during cryotherapy treatment has been well documented (Enwemeka et al., 2002; Jutte et al., 2001; Merrick et al., 1993; Myrer et al., 1998; Myrer et al., 2001; Yanagisawa et al., 2007). Skin is the first site to respond to cold exposure and its temperature decrease linearly (Chesterton, Foster, & Ross, 2002). Skin is responsible for initiating thermoregulatory responses in the muscle because muscle lacks thermal receptors. Thus, skeletal muscle tissue perfusion is a reflexive mechanism from the skin. During skin cooling, based on Fourier's law of heat conduction, deep muscle tissue loses heat mainly to the superficial muscle tissue (Bleakley & Hopkins, 2010); however, the temperature gradient between these layers is minimal, leading to smaller temperature changes than seen at the skin. Simultaneously incoming warm blood is diverted to the deeper tissues, thereby slowing down the cooling effect of the deep tissues (Pugh et al., 1960). The magnitude of change in muscle temperature is not linear. Superficial intramuscular temperature cools faster, and to a greater magnitude than deeper muscle tissues (Merrick et al., 2003; Yanagisawa et al., 2007). Finally, if the magnitude of cooling is sufficient, and not just localised to the skin, a reduction in core temperature will result. Importantly, administering cryotherapy to a greater surface area could elicit a systemic cooling effect and decrease core temperature to a greater degree than local cooling (see Figure 4).

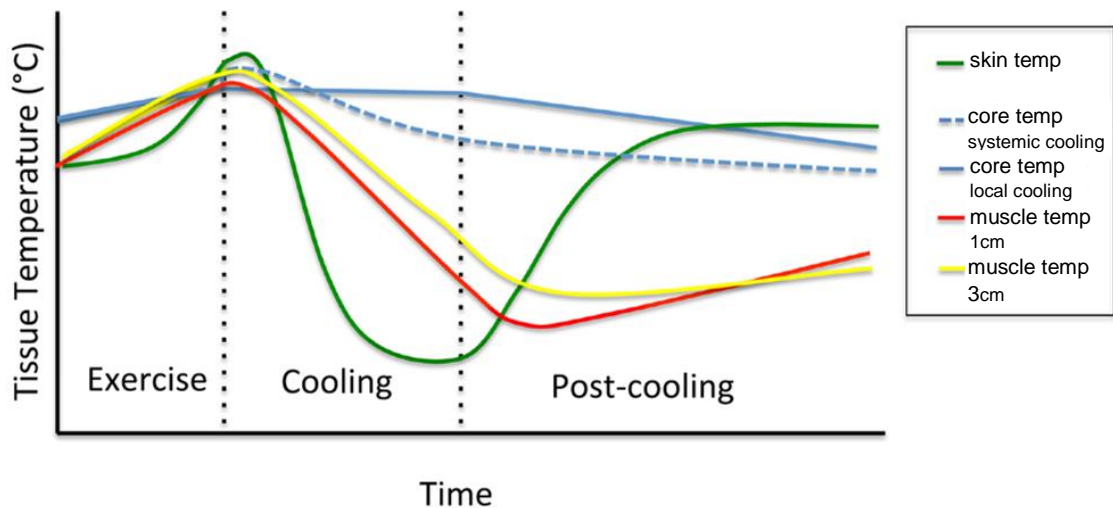


Figure 4: *Relative pattern of temperature change in different tissues during the exercise, cooling, and post-cooling period depicting that the rate of temperature change during cooling is not linear across all tissue. Exercise elevates the temperature of all tissue, dependant on exercise intensity, duration, and external environmental conditions. (Figure adapted from White & Wells, 2013).*

In order to optimise effectiveness and induce changes in blood flow, inflammation, and tissue metabolism, heat removal must occur at the target muscle and not just at the overlying skin. Reducing skin temperature alone without eliciting reductions in the deep muscle tissue or core temperature might not be enough to invoke a beneficial effect on local muscle recovery or systemic recovery following exercise. This is especially important because physiological changes following cryotherapy administered after exercise are dependent on the magnitude of tissue cooling (Costello et al., 2012a, 2012b; Mawhinney, Jones, Joo, Low, Green, & Gregson, 2013), rather than a consequence of reduced muscle blood flow, and might be of greater importance for muscle recovery than the vascular response (Swenson et al., 1996).

During cryotherapy application, the skin and superficial tissues reach significantly lower temperatures than the muscle (i.e. skin temperature values of $6.5 \pm 3.4^{\circ}\text{C}$ vs muscle temperature values of $27.8 \pm 3.5^{\circ}\text{C}$ 1 cm below subcutaneous level; Merrick et al., 2003). Thus, the skin is most prone to irreversible damage. Adverse effects can occur from exposures to low temperatures for excessive durations or very low temperatures very quickly, especially those that rapidly reduce skin temperature before muscle and core temperatures can catch up (for review see Tipton, Collier,

Massey, Corbett, & Harper, 2017). Some of these effects include nerve damage (Costello, McInerney, Bleakley, Selfe, & Donnelly, 2012b), blood acidosis (Wilcock et al., 2006), and ice burn/frostbite (Wilke & Weiner, 2003) when the cell is cooled to -10°C (Gage, 1979; Selfe, Hardaker, Whitaker, & Hayes, 2007). Thus, athletes and practitioners should understand the relationship between the patterns of tissue cooling and the significance of reducing tissue temperature to dangerous levels.

The efficacy of cryotherapy is often assessed through skin surface temperature measurements when direct measures are unavailable. Early cryotherapy literature assumed that changes in intramuscular temperature are strongly related to changes in skin temperature; however, skin temperature is a poor predictor of deep tissue temperature (Bleakley & Hopkins, 2010). Thus, reductions in skin and intramuscular temperature are not strongly correlated because the patterns of tissue cooling differ (Figure 4; White & Wells, 2013). The magnitude of change in muscle temperature is dependent on the thermal gradient between the muscle and cryotherapy medium (Merrick et al., 2003), and is inversely correlated with subcutaneous adiposity (Myrer et al., 2001; Otte, Merrick, Ingersoll, & Cordova, 2002). Subcutaneous adiposity has low thermal conductivity, creating an insulating effect (Bleakley & Hopkins, 2010). Thus, the magnitude of reduction in intramuscular temperature will be less as skin-fold thickness increases (Selkow et al., 2012; Stephens, Halson, Miller, Slater, & Askew, 2017). For this reason athletes and clinicians should take subcutaneous tissue thickness into account when aiming to achieve a specific degree of intramuscular cooling (Selkow, 2019). For example, to reduce muscle temperature by a standard amount in a heterogeneous group, Otte et al. (2002) demonstrated that an individual with a 31-40 mm skinfold thickness would have to apply ice for almost six times longer than an individual with 0-10 mm skin-fold thickness. Similarly, an individual with a 0-10 mm skin-fold thickness will achieve the same degree of intramuscular cooling as an individual with a 31-40 mm skin-fold thickness, but the cooling duration will take 10 minutes vs 60 minutes respectively (Selkow, 2019).

The magnitude of change in core temperature is primarily dependent on gender (Boehm & Miller, 2019; Kenny & Jay, 2007). In addition to anthropomorphic (body surface area, lean body mass), other gender differences exist that will affect the rate of core cooling such as the hormonal or sympathetic response. The rate of core cooling is secondarily dependent on the cooling stimulus, the amount of body surface area exposed to the cryotherapy medium (McDermott et al., 2009), and body composition (Bleakley & Hopkins, 2010; Myrer et al., 2001; Otte et al., 2002; Stephens, Halson, Miller, Slater, & Askew, 2018). For example, following exercise

when cryotherapy treatment temperature and duration were controlled for, core temperatures were significantly lower in males with a low body-fat percentage ($\leq 12.0\%$) than in those with high body fat ($\geq 18.0\%$; Stephens, Halson, Miller, Slater, Chapman, et al., 2018). Counterintuitively, it has recently been demonstrated that under hyperthermic conditions such as those occurring during maximal exercise, females cool faster than males (Boehm & Miller, 2019). Thus, although the intramuscular cooling rate is comparable across genders, the rate of core cooling is faster in women. For these reasons, cryotherapy application should be tailored specifically to the individual.

2.2.4 Mechanism of Cooling

The mechanisms of cooling are temperature dependent and reliant on vasoconstriction leading to a decrease in blood flow (Bleakley & Davison, 2009; Gregson et al., 2011; Mawhinney et al., 2013). Reducing skin temperature induces an immediate vasoconstriction reflex at the site adjacent to the cold stimulus (Johnson, Yen, Zhao, & Kosiba, 2005). During cryotherapy, vasoconstriction contributes to reduced blood flow of the underlying muscle (Ihsan, Watson, Lipski, & Abbiss, 2013; Mawhinney et al., 2013; Stanley, Peake, Coombes, & Buchheit, 2014; Thorsson, Lilja, Ahlgren, Hemdal, & Westlin, 1985; Vaile et al., 2011). Vasoconstriction redistributes blood away from the periphery and toward the core (Ihsan et al., 2013; Mawhinney et al., 2013; Song, Chelstrom, Levitt, & Haumschild, 1989; Vaile et al., 2011) in an effort to maintain core temperature (Bonde-Petersen, Schultz-Pedersen, & Dragsted, 1992; Gregson et al., 2011; Knight & Londeree, 1980; Mawhinney et al., 2013). The result is a central cascade of venous return (Bonde-Petersen et al., 1992; Vaile et al., 2011) which increases stroke volume (Stanley et al., 2014), central venous pressure (Gabrielsen et al., 1993; Johansen, Jensen, Pump, & Norsk, 1997; Mourrot et al., 2008; Park, Choi, & Park, 1999), central blood volume and flow (Song et al., 1989), and improved cardiac output (Gabrielsen et al., 1993; Park et al., 1999; for review see Wilcock et al., 2006).

Cold-induced reductions in muscle blood flow have traditionally been proposed to limit inflammatory signalling (Knight et al., 2000; Swenson et al., 1996), muscle metabolism, and subsequent secondary damage to the muscle fibres (Merrick et al., 1999). Mechanical or metabolic stress causes structural damage which initiates an inflammatory cascade (see section 2.1.2.2). Consequently, treatments to facilitate recovery have focused on attenuating inflammation. Indeed, artificially repressing the inflammatory response triggered by EIMD has been shown to result in a decreased

appearance of secondary muscle damage (Lapointe, Frenette, & Côté, 2002b). However, there is insufficient data to support the theory that cryotherapy reduces the post-exercise inflammatory cellular stress response. The effects of cryotherapy following exercise on inflammation, which can be measured via inflammatory cytokines in the blood, are conflicting. Only a few studies have demonstrated a positive effect (Guilhem et al., 2013; Pournot et al., 2011; Ziemann et al., 2012). Most studies have failed to show a beneficial effect (Fragala et al., 2015; Ingram, Dawson, Goodman, Wallman, & Beilby, 2009; Leeder et al., 2015; Peake et al., 2016; Pointon, Duffield, Cannon, & Marino, 2011b; Pointon & Duffield, 2012), and some have demonstrated a negative effect (Gonzalez et al., 2014; Jajtner et al., 2015; Roberts, Nosaka, Coombes, & Peake, 2014; Tseng et al., 2013).

Similarly, the ability of cryotherapy to reduce muscle metabolism in humans has not been established. In theory, reducing the metabolic rate of tissues within and around the muscle site experiencing structural damage decreases the oxygen requirement of the damaged tissues and reduces secondary tissue hypoxia. In the immediate period after EIMD, this effect would protect the healthy neighbouring muscle cells from the ischemic environment, thereby reducing the proliferation of secondary damage (Knight, 1995; Merrick et al., 1999). Unfortunately, the only direct evidence supporting this notion comes from animal studies (Sapega et al., 1988). One study indirectly measuring muscle metabolism in humans concluded that post-exercise cooling decreases muscle metabolic activity but did not concomitantly report intramuscular temperature (Ihsan et al., 2013). According to van't Hoff's Law, in humans, for every 10°C reduction in intramuscular temperature, a 2-3 fold reduction in the rate of chemical reaction and a 50% decrement in metabolic activity of the tissue has been proposed to occur (Brooks, Hittelman, Faulkner, & Beyer, 1971). However, it appears difficult to reduce deep tissue intramuscular temperatures in humans by 10°C for a long period of time, and temperatures rarely fall below 20°C (Bleakley & Hopkins, 2010; Merrick et al., 1993). Favourable decrements in muscle metabolism can be further challenged by the area of muscle damage, and/or by the effect of adipose tissue on heat extraction (Bleakley & Hopkins, 2010). Thus, although cryotherapy is promoted for its ability to reduce inflammation and muscle metabolism, the lack of evidence in the literature for a positive effect on either of these variables is likely due to the fact that the cryotherapy stimulus is not sufficiently reducing deep muscle temperature at the site of primary injury in the first place. As a result, neither inflammation nor muscle metabolism are reduced for a period of time adequately long enough to have any beneficial effect on recovery.

Cryotherapy is likely more effective in facilitating metabolite removal from and oxygen and substrate delivery to the recovering muscle through its ability to reduce oedema (Ihsan, Watson, & Abbiss, 2016). Vasoconstriction and blood flow redistribution decrease microvascular perfusion in the skeletal muscle, reducing the permeability of cellular, lymphatic and capillary vessels, and vessel walls (Cleak & Eston, 1992; Fridén & Lieber, 2001; Meeusen & Lievens, 1986; Swenson et al., 1996; Wilcock et al., 2006). This causes an osmotic gradient which facilitates the movement of fluids, necrotic tissue, and debris from the intracellular and interstitial spaces to the extravascular compartments (Enwemeka et al., 2002; Eston & Peters, 1999; Gabrielsen et al., 1993; Johansen, Bie, Warberg, Christensen, & Norsk, 1995; Park et al., 1999; Stocks et al., 2004) and from the extravascular space into the peripheral and central circulation (Ihsan et al., 2016; Wilcock et al., 2006). The result is a reduction in the transit distance between the capillaries and muscle fibres which may decrease mitochondrial energy production limiting ROS mediated damage.

2.2.5 Types of Cryotherapy for Exercise Recovery

Cryotherapy can be administered in a number of different ways. Local cold application, using ice, cooling pad or air pulsed cryotherapy, covers small areas and might only produce local alterations. Whole-body exposure to a cryotherapy medium, such as that occurring from CWI or whole body cryotherapy (WBC), may cause effects to the whole body and produce a greater temperature gradient for tissue cooling. While CWI is frequently used for exercise recovery, cooling jackets have recently gained popularity for their use in mitigating the rise in core temperature in warm exercise environments (Brade, Dawson, Wallman, & Polglaze, 2010; Gao, Kuklane, & Holmér, 2011; Ross et al., 2011).

2.2.5.1 Ice

During rehabilitation from injury, ice is used to relieve pain, reduce inflammation, and reduce joint oedema, promoting an earlier return to exercise (Ernst & Fialka, 1994; Knight et al., 2000). The physiological effects attributed to ice are a decrease in pain, swelling, muscle spasm, and secondary hypoxic injury (Ciolek, 1985; Knight, 1985). However, these effects following injury do not directly translate to an exercise recovery model. Literature investigating the effect of ice application following exercise has shown no effect on soreness (Gulick, Kimura, Sitler, Paolone, & Kelly, 1996; Nogueira, Felappi, Lima, & Medeiros, 2019; Yackzan, Adams, & Francis, 1984). A recent meta-analysis concluded that local cryotherapy in the form of ice does not

improve symptoms of DOMS or strength loss following exercise (Nogueira et al., 2019). Importantly, strength is decreased immediately following ice treatment (Ruiz, Myrer, Durrant, & Fellingham, 1993). Thus, returning athletes to competition immediately following cryotherapy could adversely affect their ability to perform. Since ice application is limited by duration, and the magnitude of cooling (measured at the skin) is dependent on the size of the ice medium (Janwantanakul, 2009), it is likely that a single ice treatment over a small area does not provide a sufficient cooling stimulus to elicit any beneficial effects on recovery.

Repeat applications at frequent intervals over several hours are common practice following an injury to the muscle (Knight, 1995). However, this protocol is not commonly implemented by athletes following exercise. Studies investigating repeat applications are limited and inconclusive, with some showing a positive effect at 48 hours post-exercise on the recovery of soreness (Oakley, Pardeiro, Powell, & Millar, 2013) and blood markers of muscle damage (Howatson & Van Someren, 2003) following exercise. Others have shown no effect (Howatson, Gaze, & van Someren, 2005) or an adverse effect on adaptation and symptoms of DOMS (Isabell, Durrant, Myrer, & Anderson, 1992; Tseng et al., 2013). Interestingly, one of the studies demonstrating a positive effect (Oakley et al., 2013) employed a treatment frequency that most closely resembled clinically recommended guidelines (application as soon as possible, several times a day for 15-20 minutes throughout the 72-hour recovery period; Michlovitz, 1990). The other study demonstrating a positive effect exhibited a significant decrease in CK levels at 72 hours compared with control in resistance-trained participants (Howatson & Van Someren, 2003). In contrast, the participants in a follow-up study utilising the same treatment parameters but showing no difference in CK levels, were untrained individuals (Howatson et al., 2005). Thus, ice is generally not effective in the treatment of EIMD induced by unaccustomed eccentric exercise. A more frequent application administered over a longer time period may prove beneficial. Unfortunately, athletes are not likely to comply with such a demanding treatment schedule.

2.2.5.2 Whole Body Cryotherapy

Exposing the whole body or part of the body to very cold air (-110°C to -140°C) for durations of 2-4 minutes is defined as whole body cryotherapy (WBC) or partial body cryotherapy (PBC), respectively (Banfi, Lombardi, Colombini, & Melegati, 2010; Costello et al., 2015). This cryotherapy modality is especially popular for treating symptoms such as pain, oedema, and inflammation in various chronic pathological

conditions (Hirvonen, Kautiainen, Moilanen, Mikkelsen, & Leirisalo-Repo, 2017; Missmann, Himsl, Mur, Ulmer, & Marschang, 2016). Accelerated subjective recovery of soreness following EIMD using WBC has recently been demonstrated (Bleakley, Bieuzen, Davison, & Costello, 2014; Costello et al., 2015) and the use of WBC for this purpose has gained some traction in athletic populations. Following exercise, WBC and PBC have demonstrated beneficial effects on inflammatory biomarkers (Banfi et al., 2010, 2009; Guilhem et al., 2013; Pournot et al., 2011; Ziemann et al., 2012), muscle damage (Banfi et al., 2009; Ferreira-Junior et al., 2015; Hauswirth et al., 2011; Ziemann et al., 2012), and soreness (Fonda & Sarabon, 2013; Hauswirth et al., 2011; Pournot et al., 2011; Ziemann et al., 2012). Evidence for an effect on functional recovery is mixed, with some studies concluding that WBC positively influenced muscle function or performance capacities that are otherwise detrimentally affected by exercise (Ferreira-Junior et al., 2015; Fonda & Sarabon, 2013; Hauswirth et al., 2011; Krüger, de Mareés, Dittmar, Sperlich, & Mester, 2015; Ziemann et al., 2012), while others have shown no effect (Costello et al., 2012a; Vieira et al., 2015). However, the two studies showing no effect administered treatment 24 hours after the exercise protocol (Costello et al., 2012a) or performed the functional testing too soon following WBC, at which point reduced muscle temperatures would have limited performance in the subsequent exercise (Vieira et al., 2015). Two more recent studies have shown no effect on inflammation, muscle damage or hormonal response (Krueger, Costello, Achtzehn, Dittmar, & Mester, 2019), or the ability of WBC to augment training-induced aerobic adaptations and performance (Broatch, Poignard, Hauswirth, Bishop, & Bieuzen, 2019). Overall, studies indicate that WBC may be successful in reducing soreness following exercise and returning athletes to pre-exercise strength at a faster rate than control conditions (Rose, Edwards, Siegler, Graham, & Caillaud, 2017), but there remains little evidence of clinically relevant improvements in functional recovery (Bleakley et al., 2014).

2.2.5.3 Cold Water Immersion

The majority of research investigating cryotherapy for recovery from exercise has utilised CWI. The mechanisms associated with CWI for recovery following exercise have been thoroughly investigated (Bailey et al., 2007; Bleakley et al., 2010; Broatch, Petersen, & Bishop, 2014; Cochrane, 2004; Glasgow, Ferris, & Bleakley, 2014; Ihsan et al., 2016; Poppendieck, Faude, Wegmann, & Meyer, 2013; Wilcock et al., 2006), and are believed to be associated with reduced temperature (Gregson et al., 2013, 2011; Mawhinney et al., 2013; Mawhinney et al., 2017; Peiffer, Abbiss, Watson, Nosaka, & Laursen, 2010; Peiffer, Abbiss, Nosaka, Peake, & Laursen, 2009; Roberts,

Muthalib, et al., 2015; Roberts et al., 2014), and reduced blood flow (Bleakley & Davison, 2009; Gregson et al., 2011; Mawhinney et al., 2013; Meeusen & Lievens, 1986) in the skeletal muscle. These physiological responses are heavily dependent on exercise mode, intensity, and duration. The magnitude of these effects are further understood to be dependent on water temperature, immersion duration, frequency of immersions, and mode of immersion (continuous vs intermittent; Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015; Machado et al., 2016; Vieira et al., 2016; White et al., 2014). However, the variables mentioned above are inconsistently manipulated throughout the literature. Although CWI differs from other forms of cryotherapy due to the added effect of hydrostatic pressure (see Wilcock et al., 2006 for review), which augments the effects of cold-induced vasoconstriction, blood flow (Gabrielsen et al., 1993; Gregson et al., 2011; Ihsan et al., 2013; Johansen et al., 1997; Mawhinney et al., 2013; Park et al., 1999; Stocks et al., 2004), and parasympathetic control (Mourot et al., 2008), hydrostatic pressure has no additional recovery benefit in reducing symptoms of EIMD (Leeder et al., 2015).

Immersion temperatures between 10-15°C are most beneficial for the recovery of soreness and functional recovery (Versey, Halson, & Dawson, 2013). However, immersion temperature has been a poorly controlled variable in the literature, ranging between 5-20°C (Bleakley et al., 2010; Versey et al., 2013). Some have shown no effect (Corbett, Barwood, Lunt, Milner, & Tipton, 2012; Poppendieck et al., 2013; Sellwood, Brukner, Williams, Nicol, & Hinman, 2007), or a positive effect (Anderson, Nunn, & Tyler, 2018; Dupuy, Douzi, Theurot, Bosquet, & Dugué, 2018; Rowsell et al., 2009, 2011) of varying immersion temperatures on variables associated with recovery following exercise. Dupuy and colleagues (2018) reported that only immersion in water temperatures below 15°C had a positive impact on muscle damage and inflammation as measured by changes in CK, IL-6, and CRP. Ultimately, reduced water temperature is not likely to produce additional recovery benefits (Poppendieck et al., 2013), and results in increased discomfort (Bailey et al., 2007; Heyman, De Geus, Mertens, & Meeusen, 2009; Vaile et al., 2008; Versey, Halson, & Dawson, 2011). Immersion duration is of greater importance because it is correlated with the magnitude of change in tissue temperature (Peiffer, Abbiss, Watson, Nosaka, & Laursen, 2009). Immersions of at least 10 minutes are most common in the literature (Banfi et al., 2010; Bleakley et al., 2010; Versey et al., 2013) but range between 3-24 minutes (Bleakley et al., 2010) and 3-20 minutes (Versey et al., 2013). Studies that have found a positive effect of CWI on soreness and functional recovery have utilised immersion durations of 5-15 minutes (Versey et al., 2013), 10-20 minutes (Bleakley

et al., 2010), or 10-15 minutes (Machado et al., 2015). Only one study has examined the effect of different CWI durations on the recovery of strength following cycling exercise (Peiffer, Abbiss, Watson, et al., 2009). However, no duration was found to accelerate recovery, likely because follow-up testing was performed very shortly following immersion during which time participants were too cold to optimally perform anaerobic tests. The effect of prolonged immersion duration on indices of recovery on the days following exercise in humans has not been studied. Importantly, in addition to the cold shock response associated with the initial immersion, prolonging immersion duration could result in cold incapacitation ensuing in rapid loss of fine and gross motor skills primarily in the hands and fingers as well as a loss of power and muscle coordination. If immersion duration is too long, hypothermia can occur.

Similar to ice application, a single CWI dose is likely insufficient to influence the mechanisms responsible for accelerating recovery. The most practical way to prolong immersion duration is to employ repeat exposures following a single exercise bout. Initially, repeat exposures were localised to one body part (e.g. the arm) submerged in water for 15 minutes (Eston & Peters, 1999; Meeusen & Lievens, 1986). Following eccentric exercise, repeat local immersion reduced muscle stiffness and muscle damage (as measured using CK) but did not affect soreness and strength loss (Eston & Peters, 1999). More recently, immersion of the entire body has been commonly implemented, since the thermal and physiological responses to water immersion are influenced by the amount of surface area exposed to the water (Stephens et al., 2017). Thus, full-body immersion is likely to have superior recovery effects than localised limb submersion. Intermittent whole body CWI results in a greater mean decrease in core temperature than a single session of continuous CWI (Stephens et al., 2017). However, studies on recovery indices following repeat whole body immersions have demonstrated conflicting results. Some have demonstrated improved recovery of performance (Vaile et al., 2008) and reduced soreness (Machado et al., 2017; Montgomery et al., 2008; Rowsell et al., 2009; Skurvydas et al., 2006; Vaile et al., 2008), while others have demonstrated no effect on systemic inflammation or functional recovery (Goodall & Howatson, 2008; Higgins, Heazlewood, & Climstein, 2011; Siqueira et al., 2018).

Limited research exists comparing the application of ice with CWI following exercise. Only one study demonstrated that CWI was more effective than ice at enhancing lactate recovery following exercise (Adamczyk, Krasowska, Boguszewski, & Reaburn, 2016). On the other hand, literature comparing PBC or WBC with CWI has shown that CWI is more effective than PBC or WBC in decreasing limb blood flow

(Hohenauer et al., 2018; Mawhinney et al., 2017) and accelerating recovery of muscle soreness (Abaidia et al., 2017). Research has evaluated the efficacy of CWI and WBC on performance following endurance exercise and found that WBC negatively impacted the recovery of muscle function compared to CWI, but neither intervention was more effective than a placebo (Wilson et al., 2018). In contrast, WBC was more effective than CWI at attenuating soreness and strength following resistance training, but neither cryotherapy intervention was more effective than the placebo treatment at accelerating recovery (Wilson, Dimitriou, Hills, Gondek, & Cockburn, 2019). These two studies are perfect examples of the variabilities in immersion protocols that exist throughout the literature. Although the two studies mentioned above employed varying exercise stimuli in order to examine the effects of CWI and WBC on metabolic (Wilson et al., 2018) and mechanical stress (Wilson et al., 2019), immersion temperature varied, even though both studies were completed by the same research lab. Ultimately any evidence in favour of CWI is likely due to the fact that temperature reductions from CWI are maintained for longer than from WBC (Costello et al., 2015; Costello et al., 2012a; Hohenauer et al., 2018; Mawhinney et al., 2017). Further, the thermal input associated with cooling between the two modalities is different (i.e. convective vs conductive cooling; Mawhinney et al., 2017). The relatively poor thermal conductivity of air compared to the greater conductance of tissue heat transfer/loss in water (Wakabayashi, Kaneda, Sato, Tochihara, & Nomura, 2008; Westerlund, Oksa, Smolander, & Mikkelsen, 2003), limits subcutaneous and core body cooling during WBC, and enhances these variables during CWI (Bleakley et al., 2014).

2.2.5.3.1 Effect of CWI on Blood Markers

A reduction in cellular, lymphatic, capillary and membrane permeability caused by vasoconstriction following CWI is believed to attenuate the efflux of CK. Alterations of tissue clearance due to blood-flow or function can affect the CK concentration in the blood. However, reviews have demonstrated only a small effect (Hohenauer et al., 2015; Leeder et al., 2012), or no effect (Anderson et al., 2018; Bailey et al., 2007; Bleakley et al., 2010; Fonseca et al., 2016; Halson et al., 2008; Leeder et al., 2015; Pointon et al., 2011b; Rowsell et al., 2009) of CWI on CK efflux on the days after exercise. Further still, some have reported an increase after cooling compared with the control condition (Pointon et al., 2011b; Tseng et al., 2013). Interestingly, the effect of CWI on reducing CK might be temperature-dependent, as post-exercise immersion in 15°C reduced CK at 72 hours post-exercise, whereas immersion in 5°C did not (Vieira Ramos et al., 2016). Ultimately, the weight of evidence suggests that

CWI has greater efficacy than WBC at attenuating CK following exercise (Abaïdia et al., 2017; Wilson et al., 2018).

Interleukin 6 (IL-6) is a cell signalling myokine that is released from contracting muscles into the circulatory system during exercise, is present during muscle regeneration and recovery, and has both pro- and anti-inflammatory properties (Tidball, 1995). Traditionally IL-6 has been used as a marker of acute inflammation after exercise but it has not consistently been associated with greater EIMD (Paulsen et al., 2012). Exercise-induced elevations in IL-6 are more apparent after exercise involving high metabolic stress of a large muscle mass than following single-joint exercise. No effect (Broatch et al., 2014; Montgomery et al., 2008; Vaile et al., 2007) or a moderate effect in attenuating increases in IL-6 (Peake et al., 2008; Pournot et al., 2010; Stacey, Gibala, Martin Ginis, & Timmons, 2010; Tavares et al., 2019) have been reported following CWI. A rise following CWI has also been demonstrated (Peake et al., 2016; Roberts et al., 2014), but may reflect the sustained release of IL-6 from skeletal muscle in response to glycogenolysis (Keller et al., 2001).

C-reactive protein (CRP) is hepatocyte-derived acute phase protein stimulated by increases in systemic IL-6 (Pepys & Hirschfield, 2003; Petersen & Pedersen, 2005) and utilised as a non-specific marker of inflammation (Pepys & Hirschfield, 2003; Ross, 1999). For this reason, CRP is commonly used to quantify inflammation following exercise when access to quantification of cytokine expression is unavailable. However, studies using CRP to quantify the inflammatory response following CWI have reported no effect (Banfi, Melegati, & Valentini, 2007; Halson et al., 2008; Ingram et al., 2009). Others suggest that CWI might enhance both the pro-inflammatory and anti-inflammatory responses responsible for muscle remodelling (Jajtner et al., 2015).

2.2.5.3.2 Effect of CWI on Recovery of Soreness

The most consistent effect of CWI is a reduction in the degree of perceived muscle soreness (Bailey et al., 2007; Barnett, 2006; Elias, Wyckelsma, Varley, McKenna, & Aughey, 2013; Hohenauer et al., 2015; Ingram et al., 2009; Machado et al., 2015; Minett, Duffield, Kellett, & Portus, 2012; Montgomery et al., 2008; Pointon et al., 2011b; Pointon & Duffield, 2012; Pournot et al., 2010; Siqueira et al., 2018; Yanagisawa et al., 2003). CWI has a positive effect on alleviating acute muscle soreness (Poppendieck et al., 2013). Meta-analyses have concluded that CWI had a moderate beneficial effect on alleviating DOMS at 24 hours (Hedges' $g = 0.4-0.7$), 48 hours (Hedges' $g = 0.6$), 72 hours (Hedges' $g = 0.2-0.6$), and 96 hours (Hedges' $g =$

0.6–0.7) following exercise (Bleakley et al., 2012; Hohenauer et al., 2015; Leeder et al., 2012; Machado et al., 2015). However, the overall effect of CWI on soreness is dependent on exercise mode, the degree of muscle damage, or the training status of the individual. For example, Leeder et al. (2011) demonstrated that CWI was effective in alleviating DOMS at 24 and 48 hours following high-intensity exercise but was only moderately effective at 48 hours following eccentric exercise. Similarly, Ihsan et al. (2016) concluded that CWI is limited in accelerating recovery from EIMD induced by single-joint eccentrically biased contractions and that CWI seems more effective in ameliorating effects of EIMD induced by prolonged endurance exercise or prolonged intermittent based exercise. Eccentric exercise elicits greater tissue damage and evokes a superior inflammatory response than concentric exercise. As a result, perceived soreness is likely to be greater following eccentric exercise. Thus, one can expect to experience a lesser overall effect from CWI on soreness following eccentric exercise than following concentric exercise. However, the physiological effects of CWI following different exercise types have yet to be elucidated.

Given the subjective nature of soreness, the efficacy of post-exercise CWI could be confounded by a placebo effect. Indeed, a recovery placebo administered after an acute high-intensity interval training session was proven to be as effective as CWI in the recovery of muscle strength over 48 h (Broatch et al., 2014). Further, training status is often not considered in the literature but is significant because unaccustomed exercise results in greater amounts of soreness. Studies showing a negligible effect of CWI on soreness all reported having utilised trained participants (Anderson et al., 2018; Delextrat, Calleja-González, Hippocrate, & Clarke, 2013; King & Duffield, 2009; Pointon et al., 2011a; Stanley, Peake, & Buchheit, 2012). This finding suggests that CWI is likely to have a lesser effect on reducing soreness in athletes as opposed to untrained individuals.

2.2.5.3.3 Effect of CWI on Recovery of Muscle Function

A major functional consequence resulting from EIMD is an immediate and prolonged loss of muscle strength, which is not necessarily accompanied by soreness (Cleak & Eston, 1992). The ultimate indicator of post-exercise recovery is the ability of the muscle to produce force (Minett & Duffield, 2014). Therefore, alleviating strength at a rate faster than normal could allow athletes to achieve better performance, giving them a competitive advantage over their peers. However, athletes have been unsuccessful in finding a modality to accelerate recovery of muscle strength following exercise. The ultimate goal of using CWI as a recovery modality is to enhance

subsequent performance; however, performance benefits following CWI are not consistently demonstrated across current research. Few studies have shown accelerated return to baseline strength following CWI compared with control 24-48 hours following exhaustive simulated team sports exercise (Bailey et al., 2007; Broatch et al., 2014; Ingram et al., 2009; Pournot et al., 2010), 48 hours following eccentric quadriceps contractions (Vaile et al., 2007), or 24-72 hours following drop jump exercise (Skurvydas et al., 2006). Others have shown no difference in the recovery of strength loss from CWI compared with control using the same drop jump protocol (Howatson, Goodall, & van Someren, 2009b; Siqueira et al., 2018). Similarly, Jakeman, Macrae, & Eston (2009) showed no difference in the recovery of torque from a single session of CWI compared with control performed following a plyometric jumping protocol. Importantly, the CWI protocols between the drop jump studies differed. Skurvydas et al. (2006) administered CWI (2 x 15 minutes at 15°C) immediately, 4, 8 and 24 hours following exercise; while, Howatson et al. (2008) and Siqueira et al. (2018) administered CWI (12 minutes at 15°C, 20 minutes at 10°C, respectively) immediately, 24, 48 and 72 hours post-exercise. A combination of multiple immersions within the first 24 hours as well as the prolonged duration of CWI exposure in Skurvydas et al. (2006) study was more effective in accelerating recovery of strength than the CWI protocols implemented by Howatson et al. (2008) and Siqueira et al. (2018).

The method of measuring strength throughout the literature investigating the effects of CWI varies greatly, which might further explain the discrepancy in the findings of the studies mentioned above. For example, Vaile et al. (2007) utilised peak force during an isometric squat as their strength measure, while Skurvydas et al. (2006) used a modified strain gauge to quantify strength. There are inconsistencies in the muscle groups and contraction types used for establishing an effect of CWI on the recovery of strength within the literature. One of the most informative criteria of muscle damage is a decrease in muscle force (MIVC), yet studies examining the effect of CWI on the recovery of strength have generally failed to utilise this measure. Of the few studies administering CWI and measuring recovery of muscle strength post-exercise (Bailey et al., 2007; Broatch et al., 2014; Ingram et al., 2009; Pournot et al., 2011; Skurvydas et al., 2006; Vaile et al., 2007), only two utilised MIVC (Broatch et al., 2014; Ingram et al., 2009). One administered CWI for 15 minutes in 10°C water following an acute high-intensity interval training session (Broatch et al., 2014) and found MIVC returned to baseline 48 hours following exercise in the CWI treatment group, but not in the control group; however, strength between the two groups was

not statistically different at any time point. Similarly, the other study applied two 5 minute immersions in 10°C water following 80 minutes of simulated team sports exercise and a 20-metre shuttle run test to exhaustion (Ingram et al., 2009), and found that while CWI resulted in lower decrements in strength and a near return of strength to baseline at 48 hours following exercise, there were no differences between recovery conditions for the isometric strength measures. In a meta-analysis including only tests of isometric/isokinetic knee extension or elbow flexion, CWI was not effective in improving the rate of recovery of muscle strength post-exercise (Leeder et al., 2012). Interestingly, it has recently been suggested that CWI mediated recovery of MIVC following exercise in the heat might not reflect recovery exclusively from EIMD (Ihsan et al., 2016), but might also include recovery from central fatigue (Minett et al., 2014; Pointon et al., 2011b).

Functional performance, such as sprint or cycling test performance and vertical jump assessment, are more indicative of overall muscular function and recovery from EIMD than MIVC. A handful of studies have demonstrated CWI induced improvements in functional performance in the post-exercise period following team sports and interval-based endurance exercise (Ascensão, Leite, Rebelo, Magalhães, & Magalhães, 2011; Elias et al., 2013; Ingram et al., 2009; Minett et al., 2014; Pointon et al., 2011b), but not after eccentric strength loadings (Goodall & Howatson, 2008; Sellwood et al., 2007). Similar to the effect of CWI on soreness, a single application of CWI might be insufficient to induce accelerated recovery of strength following damage to muscle contractile properties. Studies showing no effect on functional recovery did not monitor the recovery of strength beyond the first 24 hours (King & Duffield, 2009; Peiffer, Abbiss, Watson, et al., 2009; Pointon & Duffield, 2012). One study demonstrated impaired peak power (Anderson et al., 2018), but utilised trained participants and a cross-over design, separating exercise sessions by 7 days. A cross-over design should not be applied to avoid the influence of the RBE on the magnitude of muscle damage between conditions. The RBE experienced by the participants in the study (Anderson et al., 2018) was not accounted for by the authors when interpreting their results. In general, studies investigating the efficacy of CWI following repeat exercise events have not taken the potential protective effect of the RBE into account.

CWI might be effective at restoring muscle function as evident during CMJ performance. The effect of CWI on jump performance seems to be temperature-dependent as only immersion in 15°C but not 5°C improved recovery of jump height while having no accelerated effect on the recovery of strength (Vieira et al., 2016).

Others have shown no effect on jump performance using the same EIMD and CWI protocol, although there was a tendency for CWI to increase CMJ performance post-exercise, this did not reach statistical significance (Siqueira et al., 2018). Jump performance is highly dependent on the myotendinous capacity to generate power, indicating that CWI could be more effective for the recovery of stretch-shortening cycle movements to a greater extent than the recovery of isometric strength. Whether CWI has specificity to restore muscle power over strength requires further research but may be explained by the ability of CWI to reduce venous blood oxygen saturation and availability and aid in the recovery of type II muscle fibres specifically (Roberts et al., 2014), which may be preferentially damaged following eccentric exercise and are the predominant fibre type in high-velocity muscle contractions involving elevated power production (Fridén & Lieber, 2001). This is assuming that CWI specifically effects the excitation-relaxation kinetics (Favero, 1999) of skeletal muscle involved in muscle power generation.

2.2.5.4 Phase Change Material

The magnitude of change in tissue temperature is greater with cryotherapy methods that undergo a phase change (Merrick et al., 2003; Dykstra et al., 2009). Specifically, Merrick et al. (2003) demonstrated that modalities that undergo a phase change caused lower skin and intramuscular temperatures than cold modalities that did not possess these properties (ice bag: 6.5°C, skin temperature; 27.8°C, 1 cm intramuscular temperature; vs gel pack: 9.9°C, skin temperature; 29.5°C, 1 cm intramuscular temperature). Unfortunately, CWI, WBC, and ice are all limited in duration of treatment application. Further, neither CWI nor WBC undergoes a phase change. A phase change is important because it relates to a property called 'enthalpy (heat) of fusion', which is the quantity of heat required to make the material change phase (Merrick et al., 2003). Enthalpy of fusion greatly enhances the ability of a cold modality that changes phase to absorb heat. For example, even though a gel pack may be initially colder than a bag of ice, the gel pack does not change phase meaning it only experiences sensible heat loss. On the contrary, a modality that changes phase such as ice experiences a latent heat phase during the phase change (see Figure 5). Therefore, modalities experiencing the latent heat phase have an advantage over modalities capable of experiencing only a sensible heat phase by providing greater thermal storage density because during the latent phase the temperature remains constant. Thus, over time the ice will elicit a greater cooling potential than a gel pack (Knight, 1995; Merrick et al., 2003).

The duration of treatment application can be prolonged utilising phase change material (PCM) that changes phase at a temperature greater than the standard gel pack (e.g. 15°C PCM vs 0°C gel pack). The cooling effect of PCM depends on the capacity to absorb heat during periods when external heat load or body heat production exceeds heat loss. When PCM is heated (by exposure to the human body), its temperature increases and reaches the point at which it changes from solid to liquid called the phase point. The phase point will dictate the duration that the PCM can hold a given temperature. When PCMs reach their phase point, they continue to absorb heat at a constant temperature until all the material is transformed into the melted liquid phase. The duration of this process, called the latent phase of melting, is variable and dependent on the temperature gradient between skin and PCM, the PCM phase point, the area covered by PCM and the volume of the PCM (Hassabo, 2014; Tiest, Kosters, Kappers, & Daanen, 2012). For example, PCMs will melt faster if the skin is warmer, PCM with a phase point of 10°C will not hold that temperature as long as PCMs with a set point of 15°C, and a small volume of PCM will melt faster than a larger volume of PCM. Ice is the most commonly utilised PCM for exercise recovery, but its phase point of 0°C limits it from sustaining its cooling capacity for prolonged periods. Ultimately, the latent phase for a 15°C PCM is significantly longer than for a 0°C ice bag (Figure 5). Importantly, before reaching the temperature at which the latent phase of melting occurs, while PCM is in its solid 'frozen' state, it will experience temperature change defined as sensible heat. Once PCM has changed phase entirely to its melted state, it will again continue to experience temperature change (sensible heat) until it equilibrates with the environmental temperature. Cryotherapy modalities that do not experience phase change, such as a gel pack or CWI, only exhibit sensible heat loss (Figure 5).

PCM was developed by NASA in 1975, based on prototype work using liquid cooling garments by Bill Elkins, as a method for the passive control of spacesuit temperature in which severe temperatures are encountered. Since then, much of the previous human research on PCM cooling has focused on the temperature-regulating effect (Gao, Kuklane, & Holmer, 2010; Gao et al., 2011) and the cooling effect to elicit thermal comfort from heat strain (Bennett, Hagan, Huey, Minson, & Cain, 1995; Chou, Tochiara, & Kim, 2008; House et al., 2013; Kenny et al., 2011; Reinertsen et al., 2008; Zhang, 2003), and from heat strain specifically following exercise (Barwood et al., 2009; Brade et al., 2010; Purvis & Cable, 2000; Tate, Forster, & Mainwaring, 2008). Most of the research has applied PCM through pockets in vests at phase points ranging between 10-31°C, for treatment durations longer than from CWI. The

longest reported treatment duration was 90 minutes at 21°C, with no adverse or negative effects at the skin (Gao, Kuklane, Wang, & Holmér, 2012). PCM vests (24°C) are more effective at cooling the skin temperatures than 28°C vests but do not affect core temperature after exercise (Gao et al., 2011). PCM with a lower melting point might reduce core temperature more effectively.

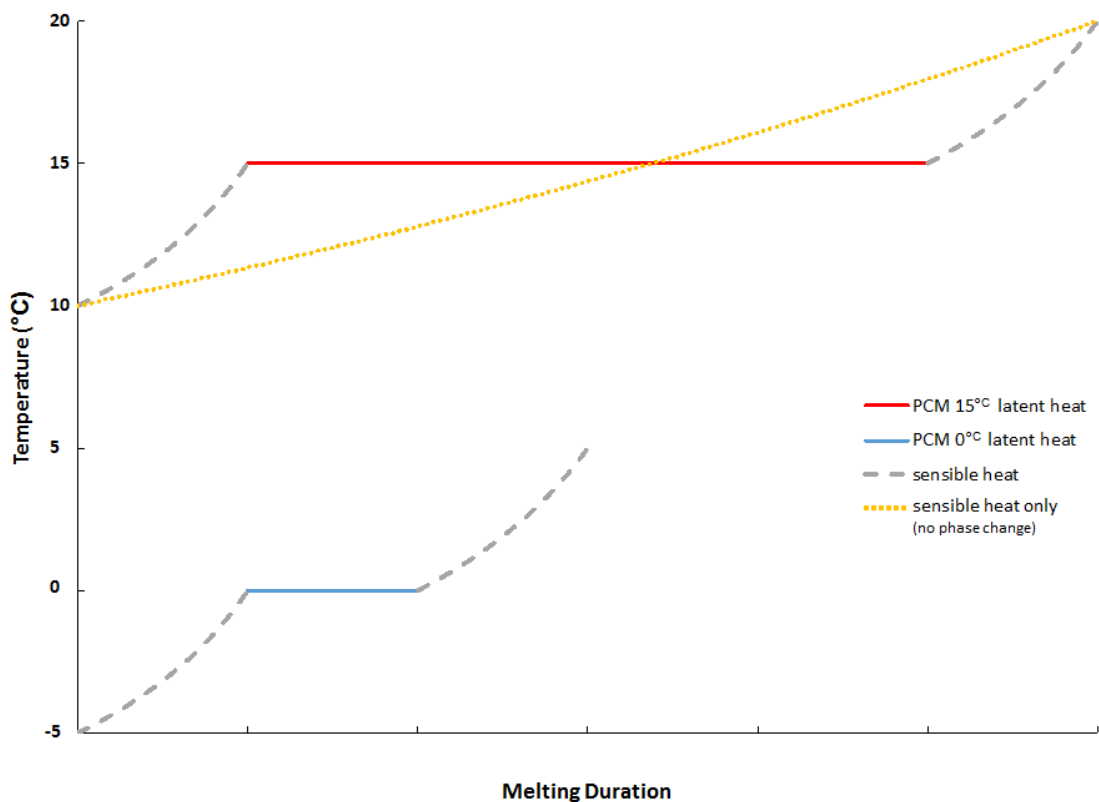


Figure 5: Sample melting pattern of PCM with a phase point of 15°C, or 0°C for comparison, as well as a material with only sensible heat properties. Latent heat period demonstrates a change in phase while maintaining a constant temperature. Latent heat phase is longer for 15°C PCM than for 0°C PCM, which is generic ice changing from solid to liquid. The 15°C PCM is compared with a reference that does not change phase (experiencing only sensible heat). For example, this can be a gel pack or CWI that initially starts at 10°C but warms up to 20°C as it equilibrates with room temperature. Melting duration is arbitrary since it is dependent on the temperature gradient between skin and PCM, the PCM phase point, the area covered by PCM and the volume of the PCM.

Applying PCM directly to the skin fitted under a garment might be more effective, less time consuming, and logistically simpler for cooling large muscles masses compared with CWI or other cryotherapy modalities, particularly if large numbers or entire

squads are seeking concurrent recovery treatment. Only one study has investigated the use of a PCM cooling vest for recovery from a metabolic challenge measuring ventilatory parameters (Hauswirth et al., 2012). They found that compared to CWI, PCM reduced physiological load in the early stage of subsequent exercise. No study has examined the effects of PCM cooling on indices of recovery from EIMD on the days after exercise. Single applications of PCM with a phase point of 15°C can provide up to 3 hours of cooling, which might improve the recovery process compared with conventional cryotherapy interventions like CWI. The ability to exploit the latent heat phase of PCM in order to maintain a steady temperature for a prolonged duration could enhance the effects of cooling on recovery and could be a practical and portable substitute to CWI.

2.2.6 Conclusion

The effectiveness of cryotherapy for the treatment of EIMD remains inconclusive. The most popular modality, CWI, offers some benefits for reducing soreness and accelerating the restoration of muscle function. However, in the literature, treatment duration and temperature vary greatly, which explains the overall equivocal evidence supporting its use for accelerating the recovery process. Further, the best available evidence supporting the use of cryotherapy for recovery stems from animal models. However, the magnitude of reduction in metabolic rate and muscle temperature achieved in animal models has not been replicated in humans. Ultimately, popular cryotherapy modalities are all limited by the duration of application. For this reason, the literature suggests that reductions in tissue temperature should be prolonged (Casa et al., 2007; Goodall & Howatson, 2008; Peiffer et al., 2009; Proulx, Ducharme, & Kenny, 2003) in order to sufficiently influence an inflammatory process that goes on for several hours (Armstrong et al., 1991), and a cascade of events that continues for several days (Lieber, 2018). Prolonging the magnitude of tissue cooling could be the missing link in mitigating the overall extent of tissue damage (Bleakley et al., 2012; Enwemeka et al., 2002; Ihsan et al., 2013; Knight, 1995; Merrick, 2002), reducing secondary muscle damage (Merrick et al., 1999; Swenson et al., 1996), and enhancing the regenerative response (Dykstra et al., 2009; Järvinen et al., 2005) following exercise. A recovery intervention capable of prolonging the cooling duration might accelerate the recovery of the signs and symptoms associated with EIMD.

3.0 GENERAL METHODS

3.1 General Methods

This chapter describes the methodological procedures common to the four experimental studies encompassing this thesis. The general methods outlined below act as a reference point for the upcoming experimental chapters. Descriptions of measures that were unique to individual studies can be found in the methods section of the relevant chapter. Institutional ethical approval was sought and granted by the Northwell Health Institutional Review Board for all investigations, while the Faculty Ethics Committee at Northumbria University granted additional ethical approval for the investigations in chapter 5 and 6. Ethical approval was granted prior to data collection for each investigation and written informed consent was sought after verbal and written detailed instructions regarding the study requirements were provided to all participants (see Appendix 9.1 and 9.2 for an example of 1. Northumbria and 2. Northwell Health informed consent, respectively).

3.2 Participant Recruitment

Recruitment was initiated through word of mouth or poster advertisement aimed at the populations described within each chapter. Healthy participants between the ages of 18-56 years were recruited for all studies. The investigations encompassing chapters 4 and 5 of this thesis recruited healthy recreationally trained female and male participants. Chapter 6 and 7 recruited only male participants, in an attempt to reduce the influence of differences in body composition on the response to cryotherapy and the hormonal response on the adaptive mechanism to exercise. The inclusion criteria for chapters 4 and 7 required participants to be acutely unaccustomed to eccentric exercise in the quadriceps, with the exception that participants in chapter 7 were required to be regularly participating in team-sport or other forms of physical exercise. Participants in chapter 5 were recruited from a pool of runners registered to complete a road marathon. Participants were familiarised with the study protocols and procedures prior to the initiation of any data collection, which are outlined in detail within each experimental chapter.

3.3 Exclusion Criteria

Participants were excluded if they had any known sensitivity to cold, allergy to cold or ice, compromised circulation or vascular disease in the legs, or had a history of musculoskeletal injury of the lower extremity within the 6 months of study participation. The use of any recovery treatments unrelated to study participation (e.g., cryotherapy, foam rolling, compression garments), nutritional supplements

(e.g., protein, antioxidants), or anti-inflammatory drugs (NSAIDS) were prohibited for the duration of each study.

3.4 Controls

Participants were instructed to limit their physical activity in the 48 hours prior to, and for the duration of the data collection period in all studies. Those participating in chapter 7 were additionally instructed to refrain from partaking in any novel eccentric exercise involving the lower limbs in the week between the two exercise bouts. Participants were not placed on any dietary restrictions and were instructed to maintain their regular eating habits. Participants were allowed to consume water ad libitum throughout each session of data collection; except not in chapter 6.

3.5 Phase Change Material Application

The experimental cryotherapy modality used throughout the course of this thesis were commercially available Phase Change Material (PCM; Glacier Tek USDA BioPreferred PureTemp PCM, Plymouth, MN, USA) packs that 'freeze' (have a latent phase) at 15°C. The Glacier Tek PCM are filled with a proprietary blend of fully hydrogenated natural fats certified by the U.S. Food and Drug Administration as food-grade chemicals such as palm oil, palm kernel oil, rapeseed oil, coconut oil and soybean oil, mixed with sodium chloride, and encapsulated in flexible plastic. This is specifically referred to as microencapsulated PCM. The PCM look like wax when in a frozen solid state and like vegetable oil when in their melted liquid state (Figure 6).



Figure 6: *Two Glacier Tek PCM packs in the frozen state (left), and in their melted state (right).*

Two PCM blocks (864 cm² area; 32.4 × 2 × 13.3 cm) fitted directly on the skin over the quadriceps on each leg were administered to each leg (Figure 7) in participants receiving the treatment condition. Participants in the control group (chapter 7), and in the group defined as indirect cooling in chapter 4, received melted room temperature PCM packs and applied them identically to the treatment condition. In chapter 5, participants in the control condition did not receive any intervention. The PCM utilised in the series of investigations in this thesis were all prepared for treatment by being frozen solid in a generic refrigerator. Preliminary testing in the lab established that the temperature gradient between the surface of the thigh skin temperature (~33°C) and the 15°C PCM results in a phase change duration of 3 hours. Additionally, the manufacturer and an independent quality association (PCM, RAL, Stuttgart, Germany), have verified that the PCM packs can maintain a constant temperature of 15°C for at least 3 hours in a thermoneutral environment until the substance is fully melted. This prompted the PCM in the treatment condition to be changed for a fresh frozen set after the initial 3 hours of wear in chapters 4 and 7. When the first set of packs had to be replaced by the participant while away from the lab, the frozen state of the second set of packs was verbally confirmed by the investigator on the second day of data collection. For participants receiving the treatment condition in chapters 4 and 7 skin temperature data were also manually inspected by the investigator to verify the cooling status throughout the entire duration of treatment. Snug-fitting athletic shorts (Eastbay brand) were worn over top of the PCM in order to hold them in place.



Figure 7: *Participant in chapter 4 wearing two PCM packs per leg, with the participant's right leg being treated with the frozen state PCM and the left leg being treated with melted PCM, and displaying the medially positioned PCM pack on each leg. The participant's right leg is receiving two frozen PCM packs applied directly to the skin over the quadriceps, which was defined as the direct cooling condition. The participants left leg is receiving two melted PCM packs applied directly to the skin over the quadriceps which was defined as the indirect cooling condition (refer to chapter 4: section 4.2.2 for further clarification, and section 4.4 for an explanation for indirect cooling). In all other chapters, participants in the treatment group received two frozen PCM packs to each leg.*

3.6 Skin Temperature (Chapters 4, 6 & 7)

Before application of PCM, in chapter 4, a thermistor probe programmed to acquire data at 1-minute intervals was taped directly to the surface of the skin over each of the thighs at approximately mid-femoral length (OMEGA Engineering, INC., Stamford, CT, USA). Before application of PCM, in chapters 6 and 7, a telemetric dermal patch skin temperature sensor sticker (VitalSense Dermal Patch, Respironics Inc., Murrysville, PA, USA) was applied directly to the surface of the skin over one thigh at approximately mid-femoral length. Data from the dermal patch was transmitted wirelessly to a data recorder found on the Sensor Electronics Module (SEM; Hidalgo Ltd, Cambridge, UK). The Respironics Sensor Electronics Module, and specifically the VitalSense dermal patch, are considered a valid and reliable tool

for the ambulatory monitoring of multiple physiological parameters such as skin temperature (Liu, Zhu, Wang, Ye, & Li, 2013). A reliability trial conducted before data collection revealed that the inter-day coefficient of variation (CV) for this protocol was 2.7%. Quadriceps skin temperature was recorded continually for the entirety of the PCM application in chapters 4, 6, and 7.

3.7 Compression Pressure (Chapters 4 & 7)

Following application of PCM, compression of one leg was measured by a pressure measuring device (Kikuhime; TT, 160 Medi Trade, Søleddet, Denmark) by placing the pressure sensor between the PCM and the anterior surface of the thigh skin (mid-point between the superior aspect of the patella and the inguinal crease). Compression was measured in both seated and standing positions. Measurements were repeated three times with the mean value recorded. The Kikuhime pressure-measuring device has previously been validated for use with compression clothing (Brophy-Williams et al., 2014). A reliability trial conducted before data collection revealed that the inter-day CV for this protocol was 11.4%. Compression pressure was recorded following application of PCM in chapters 4 and 7.

3.8 Strength: Maximal Isometric Voluntary Contraction [MIVC] (Chapters 4, 5 & 7)

Strength of the knee extensors was assessed unilaterally on both legs. The strength testing was completed on one leg and then immediately repeated on the contralateral leg. Strength was determined using an isokinetic dynamometer (chapter 4: Biodex System 2; chapters 5, 7: Biodex System 3, Shirley, NY, USA) with a leg extension attachment. Participants were seated with the trunk at approximately 90° of flexion, and a joint angle equivalent to 90° knee flexion, as assessed by a goniometer within the Biodex software and verified visually using a generic level. The lateral femoral condyle was aligned to the centre of rotation of the dynamometer. Participants were positioned for testing knee flexion according to the Biodex Multi-Joint System Setup/Operation Manual. Extraneous movement of the upper body was limited by two harnesses across the chest and the abdomen.

During the familiarisation session, participants completed the strength testing protocol twice. During the first run through of the strength testing protocol, participants were instructed to 'apply pressure' to the pad attached to the strap of the leg extension attachment but 'not to hold the contraction', in order to understand which direction they would be pushing in as well as to understand the timing of the breaks between

repetitions. This was repeated at each angle of knee flexion to be tested. During the second run through, the verbal cues were described to the participants and participants were asked to perform each isometric contraction at what they believed to be 50% of their maximal intensity for the full 5 seconds.

At each knee flexion angle being assessed, the strength testing procedure included one repetition deemed the 'warm-up', followed by two maximum isometric voluntary contractions (MIVCs). For the warm-up, participants performed one isometric contraction of the knee extensors at a self-selected intensity, but not at what they believed at the time was their maximal intensity. After a 30 second rest period, this was followed by two MIVCs, held for 5 seconds each with rests of 15 seconds between each contraction. Participants were given strong, standardised verbal encouragement for the duration of each contraction. The average of the two peak force efforts at each joint angle was recorded as the measure of MIVC (units: Newton meters [Nm]) and used for data analysis. Reliability of the MIVC torque measurement is generally high (intraclass correlation coefficients ≥ 0.85 ; Abernethy, Wilson, & Logan, 1995; Kellis & Baltzopoulos, 1995). Howatson et al. (2009a) previously analysed the inter-day test-retest reliability of MIVC measured on a fixed isokinetic dynamometer over 5 days and found MIVC to be highly reliable (intra-class correlation coefficient = 0.99; CV = 1.0%). A reliability trial conducted before data collection revealed that the inter-day CV for this protocol was 7.5%. Measures of MIVC were completed in chapters 4, 5, and 7.

3.9 Eccentric Muscle Damage Protocol (Chapters 4 & 7)

The exercise protocol was performed on the same isokinetic dynamometer that was used to measure strength. The eccentric exercise protocol was always performed immediately following strength testing without adjusting any of the settings established prior to strength testing. The exercise protocol consisted of 120 eccentric quadriceps contractions with 60 seconds of rest between sets (chapter 4: 12 sets, 10 repetitions; chapter 7: 10 sets, 12 repetitions). Target intensity was determined as 90% of previously measured MIVC at 80° of knee flexion. The range of motion was 5° (0° = full extension) to 95° knee flexion in chapter 4, and 40° to 100° knee flexion in chapter 7. Contractions were performed at 1.05 rad s⁻¹ (60° s⁻¹). Based on previous work (McHugh & Pasiakos, 2004; McHugh & Tetro, 2003), it was expected that an angular velocity of 1.05 rad s⁻¹ was sufficiently slow enough to allow participants to produce the target torque accurately and would result in significant strength loss and soreness on subsequent days following exercise. The protocol was

immediately repeated on the contralateral leg. Both legs performed the eccentric exercise, one at a time, in order to increase the likelihood of elevating the inflammatory response (Margaritelis et al., 2015). During the familiarisation session, participants completed only two sets of the eccentric exercise protocol one time, and only at an intensity that allowed the participant to understand the motion necessary to perform the exercise (< 50% of participants expected MIVC).

3.10 Muscle Soreness [DOMS] (Chapters 4, 5 & 7)

Delayed onset of muscle soreness (DOMS) in the lower limbs was assessed by having participants perform a two-legged squat to 90° knee flexion and verbally report the discomfort level for each leg using a 0 to 10-point visual analogue scale (VAS; 0=no discomfort, 10=too painful to squat to 90°).

3.11 Blood Sampling and Analysis (Chapters 5 & 7)

In chapters obtaining blood samples, blood was drawn by way of a finger prick. Blood samples were always performed prior to any activity being initiated by the participants. The fingertip was cleaned with 95% ethanol before an automatic lancet device was used to puncture the skin to draw capillary blood. The first drop of blood was removed to prevent possible contamination. A 30 µL sample of capillary blood was obtained using a 30 µL pipette (Microsafe Tubule, Safe-Tec Clinical Products, Pennsylvania, USA) for the enzymatic measurement of CK concentration. The sample was then immediately analysed (Reflotron® Plus System, Roche Diagnostics, Basel, Switzerland) using a CK test strip (Reflotron CK, Roche Diagnostics, Mannheim, Germany). A 10 µL sample was obtained in a 10 µL pipette for the immunochromatographic assay of hsCRP (Nano-Checker 710, Nano-Ditech Corporation, Cranbury, NJ, USA) using a hsCRP test strip (Nano-Check hs-CRP, Nano-Ditech Corporation, Cranbury, NJ, USA) and following the manufacturer's guidelines. The intra-sample coefficient of variation is 3.1% for the CK analyser (Horder et al., 1991), and a reliability trial conducted before data collection revealed that the inter-day CV for analysis of CK was 7.9%. The intra-sample coefficient of variation is 15% for hsCRP (Nano-Ditech Corporation, Cranbury, NJ, USA), and a reliability trial conducted before data collection revealed that the inter-day CV for analysis of hsCRP was 17.7%. Blood samples for determining the concentration of CK and hsCRP were drawn and recorded in chapters 5 and 7.

4.0 THE EFFICACY OF COOLING WITH PHASE CHANGE MATERIAL FOR THE TREATMENT OF EXERCISE-INDUCED MUSCLE DAMAGE: PILOT STUDY

This chapter has been accepted for publication:

Kwiecien SY, McHugh MP, Howatson G. (2018). The efficacy of cooling with phase change material for the treatment of exercise-induced muscle damage: pilot study. *J Sports Sci.* 36(4):407-413.

4.1 Introduction

The deleterious effects of EIMD following a bout of unaccustomed exercise, particularly when the exercise is eccentric-based, are well documented (see section 2.1.2). The magnitude and duration of the EIMD response can depend on the exercise mode, duration, and intensity of the exercise and is characterised by a reduction in strength and increased muscle soreness on the days after exercise (Clarkson & Newham, 1995; Miles & Clarkson, 1994). Timely recovery from these symptoms is therefore critical to maintain readiness to train and compete on subsequent sessions and days.

Given that EIMD is a multifaceted process, an intervention strategy for its prevention does not yet exist. The alternative is to administer an intervention to effectively treat the symptoms of EIMD after they manifest in an attempt to reduce the extent of secondary muscle damage. The most popular strategy used to facilitate recovery after exercise resulting in EIMD is cryotherapy (see section 2.2). Cryotherapy relates to the cooling of an area in the attempt to induce clinically relevant reductions in body temperature (see section 2.2.3). The reductions in skin, muscle, and core temperature associated with cryotherapy are purported to alleviate some of the physiologic and functional deficits associated with EIMD (see section 2.2.4). Ultimately the precise mechanisms through which cryotherapy might accelerate recovery following exercise are not well understood. Recently, using a contralateral cryotherapy model, Allan et al. (2017) demonstrated that administering cryotherapy to a single limb exerted adrenergic control, resulting in a systemic response evident in the untreated limb. This evidence established the basis for bilateral research designs with true control conditions when investigating the cold-induced post-exercise response.

The most popular cryotherapy strategy used to facilitate recovery after strenuous exercise resulting in EIMD is CWI (see section 2.2.5.3) because it offers a quick, simple, and if required, whole body intervention. Previously a moderate effect on soreness, and no effect on strength, with some benefit for power restoration has been demonstrated following CWI (Bleakley et al., 2012; Leeder et al., 2012; Versey et al., 2013). Although the average immersion duration across studies was 12.5 ± 5.6 minutes within a temperature range of 10-15°C (Bleakley et al., 2012), there remains great variability in the duration and temperature of immersion protocols (Bleakley et al., 2012; Leeder et al., 2012; Poppendieck et al., 2013; Versey et al., 2013), and no consensus for optimal treatment criteria. Ultimately CWI is limited by its treatment

duration as it can only be applied for short periods due to contraindications from spending extended periods immersed in cold water, athlete tolerance to the intervention and logistical issues surrounding delivering such an intervention in applied scenarios.

To date, following exercise, prolonging the duration of cryotherapy has only been achieved through repeated exposures (Skurvydas et al., 2006). A cryotherapy modality capable of delivering cooling for one period of a prolonged duration might overcome the limitations associated with other cryotherapy modalities outlined in section 2.2. The duration of cryotherapy application can be prolonged using PCM because PCM has the capability to maintain a constant temperature for a prolonged duration (see section 2.2.5.4). Conceptually, the recovery process could be enhanced using PCM given that the cold application time would be longer than conventional cryotherapy methods, like CWI, allowing for cooler temperatures to be maintained for a longer period. When applied to exercise paradigms, several studies have reported that PCM with a melting point $\geq 0^{\circ}\text{C}$ administered to the core in the form of a vest resulted in a reduction in core temperature (Tate et al., 2008) and skin temperature (Barwood et al., 2009; Brade et al., 2010; Purvis & Cable, 2000), compared to control conditions. However, the local application of PCM set to melt at 15°C in an exercise recovery paradigm has not been investigated.

As a first step to understanding the potential of prolonging the duration of cooling this chapter aimed to establish the efficacy of 6 consecutive hours of PCM cooling, while being mindful of the evidence that implicates a systemic response from unilateral cryotherapy treatment (Allan et al., 2017), by administering direct- and indirect- PCM cooling to a single muscle group as compared to control in the recovery from EIMD. Consequently, the purpose of this pilot study was to establish the efficacy of a novel cooling strategy (15°C PCM) as a recovery intervention following a bout of isolated eccentric exercise. The overall aim of this thesis is to investigate the efficacy of PCM, capable of prolonging the duration of cryotherapy exposure, as an alternative cryotherapy modality for accelerating recovery following strenuous exercise. Therefore the findings of this chapter will address the specific aim of this thesis by establishing whether prolonging the duration of cooling is capable of accelerating recovery of indices associated with EIMD induced through mechanical stress. The findings might also provide further evidence as to whether local unilateral cooling exhibits a similar systemic effect as was evident in the model implemented by Allan

et al. (2017). It was hypothesised that prolonged PCM cooling would alleviate strength loss and soreness on the days following the eccentric exercise.

4.2 Methods

4.2.1 *Participants*

A power calculation was conducted to determine an adequate sample size for this investigation. Using the findings of previous work documenting strength loss and pain following similar bouts of eccentric exercise of the quadriceps (McHugh & Pasiakos, 2004; McHugh & Tetro, 2003) and hamstrings (McHugh & Nesse, 2008), it was estimated that in order to detect significant changes strength loss would average 20% over the 3 days after exercise in the control leg. Thus, it was estimated that there would be 80% power to detect a 14% difference in strength loss between treatments at an alpha level of 0.05 with a total of 12 participants.

Consequently, six males and two females (mean \pm SD; age, 37 ± 12 years; height, 177.1 ± 9.3 cm; body mass, 76.4 ± 9.7 kg) volunteered to participate. Participant recruitment was based on the details described in section 3.2 and the exclusion criteria described in section 3.3. Participants adhered to the controls described in section 3.4 throughout the duration of data collection. The institutional research ethics committee, in line with the Declaration of Helsinki, approved all procedures.

4.2.2 *Experimental design*

Participants reported to the laboratory for a total of six days; one familiarisation session, one baseline testing session which included the eccentric exercise protocol, and four consecutive days following the exercise to assess quadriceps strength and muscle soreness. On the first visit, participants were familiarised with all testing procedures. Familiarisation occurred 7 days prior to baseline testing. On the day of baseline testing, participants had their quadriceps strength, and soreness assessed of both limbs prior to performing an eccentric exercise protocol of the knee extensors. The same examiner conducted the tests of MIVC and the exercise protocol, using standardised verbal commands to ensure consistency. Participants were randomised in a counterbalanced order to initiate the eccentric exercise on either the dominant or non-dominant leg. The exercise protocol was immediately followed by the application of frozen (direct cooling) PCM to the quadriceps of one leg and room temperature (indirect cooling) PCM to the other leg (see section 4.2.3). Five months later the participants returned to the laboratory and the protocol was repeated bilaterally with

room temperature PCM applied to both legs. The 5-month delay was implemented in order to ensure that the RBE did not impact the damage response (Nosaka, Newton, & Sacco, 2005). Strength and soreness values for the control treatment were averaged across both legs. The experimental design is summarised in Figure 8.

Leg 1	Time	Leg 2	
Strength Test	15 MIN		
Eccentric Exercise	30 MIN		
	BASELINE	15 MIN Strength Test	
		30 MIN Eccentric Exercise	
PCM Room Temp INDIRECT COOLING		6 HRS	6 HRS PCM Frozen DIRECT COOLING
Remove PCM		Remove PCM	
	Day 1		
Strength Test	Day 2	Strength Test	
Soreness	Day 3	Soreness	
	Day 4		
5 MONTHS LATER			
Strength Test	15 MIN		
Eccentric Exercise	30 MIN		
	BASELINE	15 MIN Strength Test	
		30 MIN Eccentric Exercise	
PCM Room Temp CONTROL		6 HRS	6 HRS PCM Room Temp CONTROL
Remove PCM		Remove PCM	
	Day 1		
Strength Test	Day 2	Strength Test	
Soreness	Day 3	Soreness	
	Day 4		

Figure 8: Schematic representation of the experimental protocol on both legs showing the sequence of events from baseline strength measures, to the eccentric exercise, to frozen PCM application on one leg (direct cooling) and room temperature PCM on the contralateral leg (indirect ‘systemic’ cooling), to strength and soreness assessments for 4 days after the isolated quadriceps eccentric exercise; followed by repeating the protocol with melted PCM on both legs (control) 5 months later. This was followed by a 5-month break to remove the effect of the RBE. The experimental protocol was then repeated with room temperature PCM applied to both legs after eccentric exercise (control).

4.2.3 Phase Change Material Application

Upon initial completion of the eccentric exercise protocol, participants immediately applied frozen 15°C PCM to the quadriceps of one leg (direct cooling), and room temperature PCM to the other leg (indirect cooling) for a total of 6 hours. The direct cooling frozen (15°C) PCM was always applied to the limb that completed the exercise protocol second in order to minimize the time between exercise cessation and the direct cooling treatment condition. Upon completion of the exercise protocol 5 months later, participants applied room temperature PCM to both legs (control) for a total of 6 hours. Thus, there were three treatments: leg directly treated with 15°C PCM packs (defined as direct cooling), leg receiving indirect cooling treated with room temperature PCM packs (defined as indirect 'systemic' cooling) contralateral to the leg receiving 15°C PCM packs, and legs tested 5 months later and both treated with room temperature PCM packs (defined as control). For complete PCM application procedures, please refer to section 3.5 and Figure 7.

4.2.4 Strength Assessment

Strength was tested at each of 30°, 50° and 70° knee flexion. For complete strength testing procedures, please refer to section 3.8.

4.2.5 Eccentric Exercise Protocol

The eccentric exercise protocol consisted of 120 eccentric quadriceps contractions (12 sets, 10 repetitions), with the range of motion set at 5° (0° = full extension) to 95° knee flexion. For thorough detail of the eccentric exercise protocol, please refer to section 3.9.

4.2.6 Soreness Assessment

For procedures to assess muscle soreness, please refer to section 3.10.

4.2.7 Skin Temperature

For procedures to assess skin temperature, please refer to section 3.6.

4.2.8 Statistical Analysis

All data are presented as mean \pm SD. Strength at each of the three test angles was expressed as a percentage of pre-exercise strength to remove the effect of inter-individual variation in knee extension strength. Strength prior to the initial eccentric exercise bout was compared to strength prior to the eccentric exercise performed 5

months later to ensure there was no systematic change over time that might have affected susceptibility to EIMD. The effect of PCM cooling on strength loss on the days after eccentric exercise was assessed using $3 \times 3 \times 4$ treatment by angle by time repeated measures analyses of variance (ANOVA). The three levels for the treatment factor were direct cooling, indirect cooling and control. The three levels for the angle factor were 30° , 50° and 70° knee flexion. The four levels for the time factor were day 1, day 2, day 3, and day 4 after the eccentric exercise. The effect of PCM cooling on soreness on the days after eccentric exercise was assessed using 3×4 treatment by time repeated measures ANOVA. Since it was possible that cooling one leg might have affected the damage response in the contralateral leg, it was hypothesised that the magnitude of strength loss and soreness for the indirect cooling treatment would lie between the responses for the direct cooling and control treatments. Therefore, the linear contrast for the treatment effects was reported in addition to the main treatment effect as recommended by Atkinson (2002). The average skin temperature during the 6 hour PCM treatment was compared between treatments using a repeated measures ANOVA.

Normality of all data sets was examined using the Shapiro-Wilk test. Mauchly's test was used to assess assumptions of sphericity and, where necessary, Greenhouse-Geisser corrections were used. Significant interactions of temperature, soreness or strength loss between the three treatments were followed up using Fisher's least significant difference test for pairwise comparisons. Where appropriate, Cohen's d ES were calculated to provide magnitude of effects; with the magnitude of effects considered either small (0.20-0.49), medium (0.50-0.79), and large (>0.80 ; Durlak, 2009). The upper and lower limit of 95% confidence intervals (CI) for least significant difference are reported where relevant. Statistical analyses were performed using SPSS v.21 (IBM, Armonk, NY, USA) and a P-value of less than 0.05 was considered statistically significant.

4.3 Results

4.3.1 Skin Temperature

There was no drift in temperature over the 6 hour cooling period. There was a treatment effect for skin temperature (Table 1; $F = 280.5$, $P < 0.0001$, $ES = 13.66$, direct cooling CI: 21-23°C, indirect cooling CI: 30-32°C, control CI: 30-32°C).

Table 1: Average skin temperature over 6 hours and temperature at hour 6 post-exercise intervention for each treatment condition. Values are mean \pm SD.

	6 Hour Average (°C)	Temperature @ 6 Hours (°C)
Direct Cooling	21.9 \pm 1.0	21.7 \pm 0.7
Indirect Cooling	31.7 \pm 1.4	31.2 \pm 1.4
Control	31.3 \pm 1.3	31.3 \pm 1.8

4.3.2 Strength

Average quadriceps strength expressed as a percentage change from baseline for the 4 days following the exercise intervention showed a main effect of time ($F = 7.0$, $P = 0.017$, $ES = 1.99$; Figure 9), indicating the presence of muscle damage following the isolated eccentric exercise. Overall recovery of MIVC was independent of treatment with no group ($P = 0.089$) or interaction effect observed ($P = 0.645$). However, the overall treatment effect showed a significant linear trend ($F = 26.1$, $P = 0.018$, $ES = 3.86$); indicating a quicker reduction in the force loss in the direct cooling PCM treatment group vs the control group ($F = 9.4$, $P = 0.018$, $ES = 2.32$, direct cooling CI: 94.9-107.3%, control CI: 86.4-98.7%), but not vs the indirect cooling group ($P = 0.086$). Over time, the treatment effect was not different between test angles ($P = 0.589$) with no interaction effect observed ($P = 0.537$). Additionally, strength loss (averaged across all treatments) was not different between test angles ($P = 0.272$). Although there was a significant angle*time interaction ($F = 3.5$, $P = 0.006$, $ES = 1.42$) because strength at 30° appeared to be less than strength at 50 and 70° on day 3, post hoc analysis revealed these differences were not significant ($P = 0.282$).

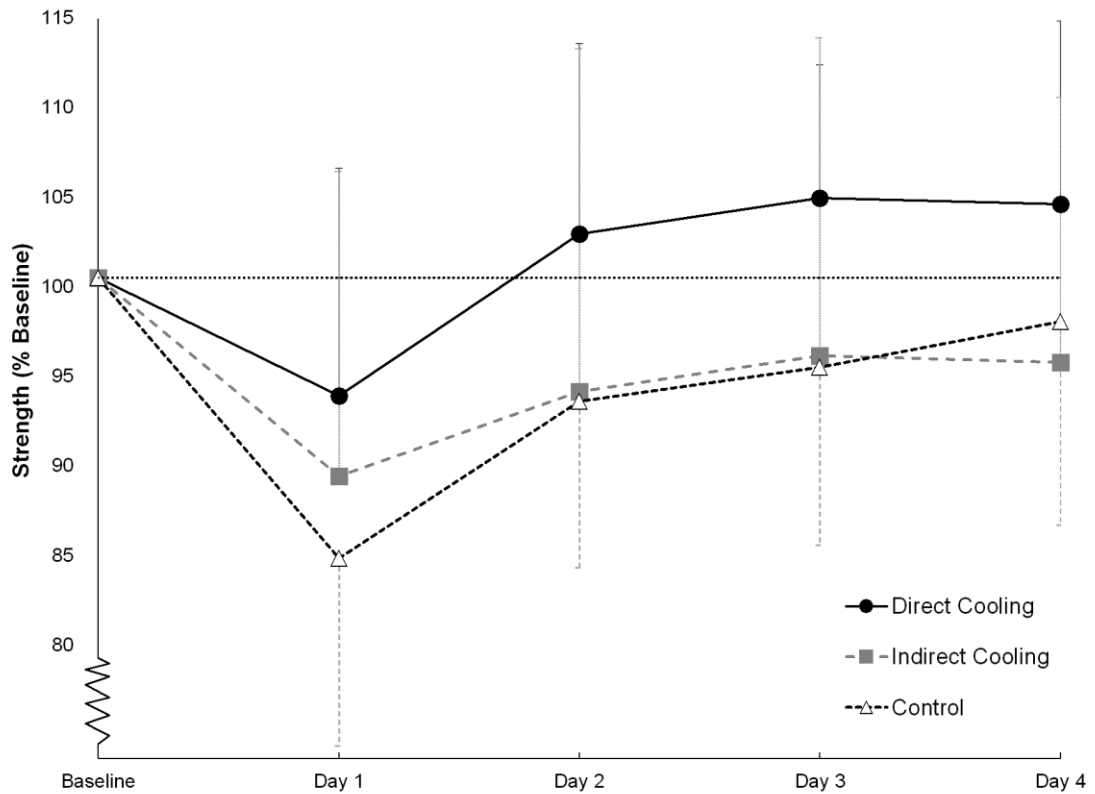


Figure 9: Isometric strength loss of the quadriceps (averaged across all three test angles and presented as a percentage change from baseline strength loss) at baseline and over the 4 days following the isolated quadriceps eccentric exercise in the direct cooling, indirect cooling and control conditions. Values are mean \pm SD. Strength was reduced over the 4 days following exercise ($P = 0.017$), with a significant linear treatment effect ($P = 0.018$) indicating accelerated recovery of strength in the direct cooling PCM treatment group vs the control group.

Baseline knee extension strength in the direct and indirect cooling legs was well reproduced 5 months later (Table 2; treatment effect: $P = 0.475$; direct cooling vs control: $P = 0.854$; indirect cooling vs control: $P = 0.994$). At baseline, there was a significant main angle effect ($F = 117.8$, $P < 0.0001$, $ES = 8.21$); peak knee extension strength occurred at 70° (281 ± 69 Nm) with lower torques at 50° (230 ± 58 Nm; $F = 67.7$, $P < 0.0001$, $ES = 6.21$) and 30° (162 ± 44 Nm; $F = 125.2$, $P < 0.0001$, $ES = 8.45$), but this was not different between treatments ($P = 0.475$). Average torque during the eccentric exercise was not different between the direct cooling (241 ± 53 Nm), indirect cooling (240 ± 52 Nm) and control conditions (251 ± 41 Nm; $P = 0.165$) conditions. Net torque data for each day of data collection are presented in Table 2 to demonstrate the return of function following the exercise protocol.

Table 2: Isometric strength (MIVC) of the quadriceps averaged across all 3 test angles for each treatment (direct cooling, indirect cooling, control) reported as net torque values (Nm) on each day after eccentric exercise. Values are mean \pm SD.

	Direct Cooling (Nm)	Indirect Cooling (Nm)	Control (Nm)
Baseline	220 \pm 60	228 \pm 60	223 \pm 51
Day 1	205 \pm 67	197 \pm 46	189 \pm 54
Day 2	223 \pm 64	212 \pm 61	208 \pm 57
Day 3	228 \pm 62	216 \pm 58	213 \pm 54
Day 4	226 \pm 62	214 \pm 50	220 \pm 64
<i>Time Effect</i>	P < 0.0001	P = 0.113	P = 0.157

4.3.3 Soreness

Soreness was elevated following the eccentric exercise ($F = 24.9$, $P < 0.0001$, $ES = 3.78$), but recovery of soreness was independent of treatment with no group ($P = 0.071$) or interaction effect observed ($P = 0.245$). However, the overall treatment effect showed a significant linear trend for soreness ($F = 7.5$, $P = 0.029$, $ES = 2.07$; Figure 10); indicating a more rapid recovery of soreness in the direct cooling PCM treatment group vs the control group ($F = 7.5$, $P = 0.029$, $ES = 2.07$, direct cooling CI: 0.025-1.6, control CI: 1.3-2.3), but not vs the indirect cooling group ($P = 0.116$).

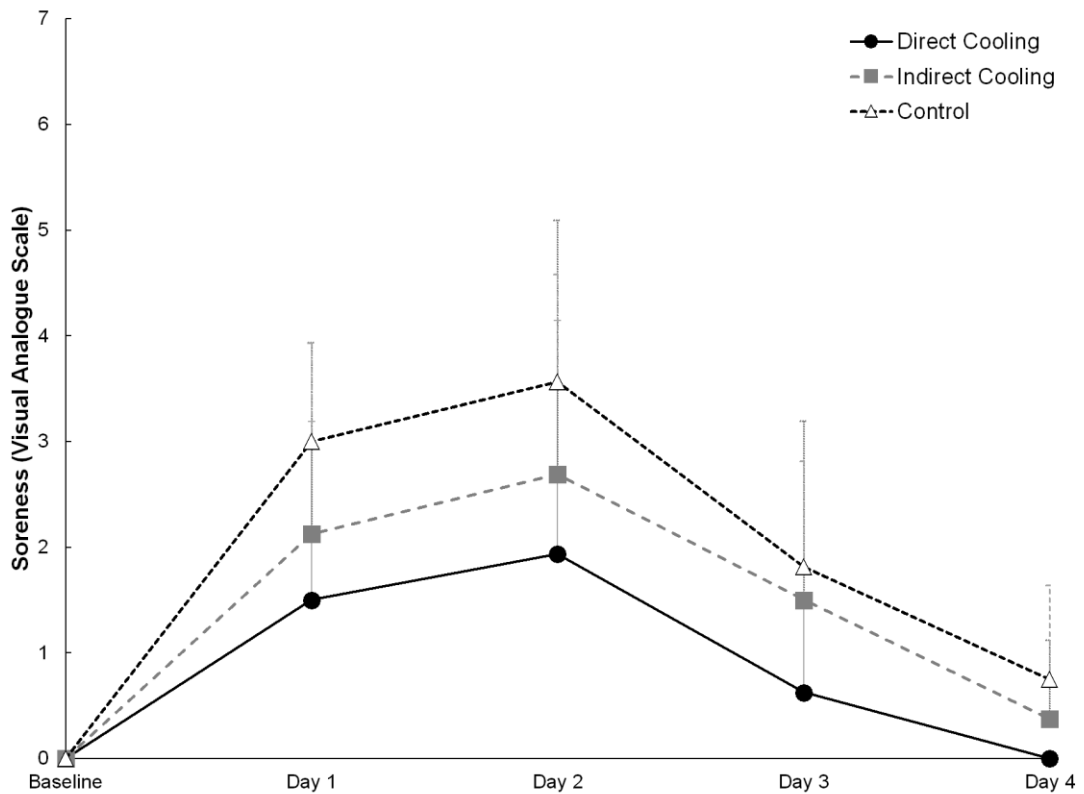


Figure 10: Subjective reports of quadriceps soreness on a 0-10 visual analogue scale (VAS; 0 = no discomfort, 10 = too painful to squat to 90°) in the direct cooling, indirect cooling and control conditions at baseline and over the 4 days following the isolated quadriceps eccentric exercise. Values are mean \pm SD. Soreness was increased following the exercise in all treatment groups ($P < 0.0001$). However, a significant linear treatment effect was evident ($P = 0.029$) indicating a more rapid recovery of soreness in the direct cooling PCM treatment group vs the control group.

4.4 Discussion

The primary purpose of this study was to test the efficacy of PCM cooling for improving recovery from exercise resulting in EIMD, in order to establish a proof of concept for future studies. The present study was the first to administer a single cryotherapy treatment for a prolonged duration. The results indicate that 6 hours of continuous direct cooling using 15°C PCM mitigated strength loss and soreness on the days following the eccentric exercise compared with a control condition. Despite greater reductions in quadriceps strength loss and muscle soreness in the direct cooling condition, no significant differences in the magnitude of effect on strength and soreness were observed between direct and indirect cooling conditions. However, the

strength and soreness values for the indirect cooling condition fell between the direct cooling and control conditions, and there was a significant linear trend for both strength and soreness. These findings suggest that the direct application of PCM cooling to one leg will systemically influence the contralateral limb receiving no cooling (indirect cooling).

This is the first study to have successfully administered a local cryotherapy modality for a prolonged duration. Previously, the longest total treatment duration included in the systematic review by Bleakley et al. (2012) involved a total treatment duration of 120 minutes (2 x 15-minute immersions in 15°C separated by 10 minutes, performed immediately post-exercise, and repeated at 4, 8 and 24 hours; Skurvydas et al., 2006). Skurvydas et al. (2006) demonstrated beneficial effects from CWI vs control for recovery of both soreness and strength on the days after performing 100 drop jumps. Their findings support the need for an increased cryotherapy duration in order to improve effectiveness on indices of recovery. However, the practicality of delivering multiple repeat CWI treatments in the initial 24 hour window after exercise can be challenging. By contrast, the present study demonstrated that wearing a pair of shorts fitted with PCM packs successfully provided a prolonged duration of targeted cooling while accelerating recovery of strength and soreness on the days following eccentric exercise. Thus, PCM cooling might be a practical alternative to other forms of cryotherapy.

The intended purpose of a prolonged period of PCM cooling was to intervene during the period of injury proliferation. The benefits of cryotherapy for accelerating recovery after EIMD are purported to be due to a reduction in temperature at the muscle susceptible to secondary damage, which might lower metabolic demand and limit the proliferation of muscle damage following exercise (see section 2.2.4 for review). In the present study, the application of 15°C PCM to the quadriceps reduced skin temperature to 22°C for the entire treatment duration. By comparison, post-exercise CWI at 10°C for 20 minutes reduced skin temperature to 22°C immediately upon cessation of immersion (Minett et al., 2014). In the same study the CWI intervention hastened the recovery of MIVC 24 hours post-exercise compared to control. Thus it is possible that reducing skin temperature for the 6 hours of cooling in the present study limited the proliferation of EIMD to a greater degree compared with 20 minutes of immersion in the Minett et al. (2014) study. Similarly, Mawhinney et al. (2013) immersed athletes in 8 or 10°C water for 10 minutes post-exercise. The reduction in skin temperature upon cessation of immersion, taken halfway between the two

immersion conditions, was $\sim 23^{\circ}\text{C}$ in their study. The authors also measured femoral artery blood flow (duplex ultrasound) and concluded that no differences were observed between cooling conditions in terms of the magnitude of effect on blood flow. Hence they concluded that the effectiveness of cryotherapy in the treatment of EIMD is dependent on its ability to reduce muscle temperature and not muscle blood flow. Although it cannot be inferred directly in the present chapter, reduced skin temperature during cryotherapy treatment might reflect reduced blood flow within the limb (Ihsan et al., 2013). Thus it is possible that the reduction in skin temperature in the present study might have translated to a reduction in blood flow, but ultimately the mechanisms through which PCM mitigated EIMD are likely related to the prolonged reduction in temperature of the exercised limb.

In the present study, the findings for the direct PCM cooling condition, that strength over the 4 days after eccentric exercise averaged 101% of baseline and soreness averaged 1.0 on a 10-point scale, indicate that direct PCM cooling minimised the extent of muscle damage. Importantly, although not significant, there was an overall trend for a significant main effect of treatment on both strength loss ($P = 0.089$) and soreness ($P = 0.071$). Furthermore, there was a significant linear trend for both strength and soreness and the corresponding strength and soreness values were 94% and 93% for strength and 1.7 and 2.3 for soreness, respectively, for the contralateral leg (indirect cooling) and control (5 months later) treatments. These results support the findings of Allan et al. (2017), who implemented a contralateral CWI study design, immersing only one leg, and demonstrated that a systemic response was evident in the skeletal muscle of the non-immersed limb. Similarly, Wolf, (1971) previously suggested that a centrally induced effect occurs as evident by a decrease in the intramuscular temperature of the contralateral extremity after cooling the ipsilateral side to 10°C for 15 min. The contralateral design of the present study allowed us to assess the potential systemic impact of the direct PCM cooling leg on the indirect cooling leg. However, this design overlooked the possibility of the RBE in the control condition when the exercise was repeated 5 months later. Ultimately the use of the control treatment was not a limitation as symptoms of EIMD are consistent within participants when a damage protocol was repeated a few months later (Nosaka et al., 2005).

A recent meta-analysis indicated that compression garments worn for recovery purposes can facilitate the return of strength and power following exercise (Hill, Howatson, van Someren, Leeder, & Pedlar, 2014b). Since this study has indicated

potential efficacy, the lack of measurement of compression in this study was a confounding factor. Although the same shorts fitted with PCM packs were worn on all test occasions, in the initial session, the direct cooling condition required the PCM to be frozen while the indirect cooling condition required the PCM to be melted. This difference between the frozen and melted state could have affected the degree of compression on the thigh. In order to determine the effect of compression during PCM application, chapter 7 will utilise a pressure monitoring device to measure the degree of pressure exerted by the garments. Additionally, in the present study, room temperature PCM was not applied to the leg receiving indirect cooling until the direct cooling leg completed the eccentric exercise. Thus, the direct cooling leg received PCM treatment and compression immediately after the eccentric exercise while the indirect cooling leg did not receive compression until approximately 35 minutes following exercise. The possibility that this delay in the application of compression affected subsequent symptoms was assessed by comparing strength loss and soreness between both legs after the control session (5 months later). In the control session, when room temperature PCM packs were applied to both thighs, the application of compression shorts was also delayed by 30 to 40 minutes in one leg compared with the contralateral leg. However, there was no difference in soreness ($P = 0.227$) or strength loss ($P = 0.787$) between legs on the days after the eccentric exercise in the control condition. Hence, the delay in the application of compression caused by the study design was not a confounding factor.

While the present study sets the stage for larger studies on the potential benefit of PCM cooling, it was not without limitations. Firstly, although a sample of eight participants was sufficient to indicate a benefit of PCM in accelerating recovery of strength and soreness, there was insufficient power to detect a difference between the control and indirect cooling treatments. Hence the sample size was insufficient to determine whether the direct PCM cooling condition caused a systemic effect in the indirect cooling limb and it was not possible to conclude whether direct cooling to one leg had a systemic effect in the contralateral leg; but the treatment effect on strength and soreness evident in the indirect cooling group indicates that this was a possibility. Secondly, quadriceps strength in the present study was measured at 30, 50, and 70°, since peak knee extension torque occurs at around 70°, with lower values occurring at 50° and 30°. In the present study, strength loss was not different between 70°, 50° and 30° ($P = 0.272$), and the effect of PCM cooling on strength loss was not different between test angles ($P = 0.537$). However, Child, Saxton, & Donnelly, (1998) had previously demonstrated greater strength loss at short muscle lengths (20° compared

with 100°) after 75 maximal eccentric contractions of the knee extensors. Lower knee extension strength has also been demonstrated at 110° (McHugh & Tetro, 2003). Therefore, strength measures at angles less than 30° or greater than 100° may be necessary to see a clear effect of muscle length on strength loss following eccentric exercise. Thirdly, familiarisation is established as being a potential contributing source of error to the testing of muscle function. Despite the participants being familiarised to the strength testing protocol in the present study, it was possible that participants could have experienced improvements in their baseline strength between the initial treatments and the control data collection periods. However, this was not the case as strength prior to the initial eccentric exercise bout was compared to strength prior to the eccentric exercise performed 5 months later, and there was no difference in absolute baseline strength values between exercise sessions. Finally, since both males and females were included, the group was not fully homogenous. In order to address these limitations, the subsequent chapter will aim to establish the efficacy of PCM cooling in a larger sample of participants, with a between-groups design to negate the potential confounding influence of a systemic effect.

This study demonstrated that direct PCM cooling was effective in accelerating recovery of soreness and strength in a single muscle group EIMD model of mechanical nature. The results further indicate that 6 hours of direct PCM cooling was well tolerated and that the prolonged PCM cooling duration was a practical and portable cryotherapy method. However, the mechanisms through which PCM might mitigate EIMD were not examined in the present study and the results cannot be directly applied to a metabolic stress model or to a training environment where multiple muscle groups are stressed. In this regard, the chapter 5 will administer cryotherapy following the completion of a marathon run.

4.5 Conclusion

This was the first examination of the prolonged application of PCM as a recovery modality from the signs and symptoms of EIMD. These data suggest that direct PCM cooling accelerated recovery of muscle function after EIMD of mechanical nature and reduced the soreness associated with muscle damage. These data also indirectly provided some evidence to support cryotherapy exhibiting both local and systemic effects; however, further direct evidence is required to support this finding. In conclusion, the data from the current study provide evidence that PCM can be utilised effectively as a recovery modality following eccentric exercise understood to elicit mechanical stress of the muscle. Given this evidence, and the practical utility of a

wearable garment to deliver prolonged cooling to muscles following exercise, a larger study to address the effect of PCM cooling on additional markers of EIMD in a larger sample, encompassing a between participant's design and using a passive recovery control group will be conducted in the subsequent chapters.

4.6 Perspectives

This chapter aimed to establish the efficacy of cooling the quadriceps for a prolonged duration (6 hours) using PCM following eccentric exercise performed by the quadriceps involving high mechanical stress. This chapter administered direct PCM cooling to the quadriceps of one leg, while a placebo condition of room temperature PCM were simultaneously applied to the contralateral leg during the 6 hour recovery period. Since following the initial exercise session it became apparent that the placebo condition might have benefited from a systemic effect of cooling, the study protocol was repeated 5 months later, and both legs received the control condition. Thus, recovery of strength and soreness were assessed following application of the 3 treatment conditions identified as: direct cooling, indirect cooling, and control. In this chapter, prolonging the duration of cooling using PCM effectively accelerated recovery of strength loss and soreness in the quadriceps compared to control. Importantly, the prolonged duration of cooling in this study was well tolerated by all participants, and no adverse events were reported. These findings addressed the first aim of the thesis, which was to establish the efficacy of PCM as an alternative cryotherapy intervention for recovery from EIMD.

This study supports the findings of previous investigations that utilised CWI to successfully reduce muscle soreness following exercise (see section 2.2.5.3.2 for review). However, unlike previous work investigating the effects of cryotherapy on the recovery of strength that generally have failed to demonstrate a beneficial effect (see section 2.2.5.3.3 for review), this study demonstrated that prolonged PCM cooling was effective in accelerating recovery of muscle strength following exercise of mechanical nature. Although this chapter demonstrated that recovery of both strength and soreness were accelerated by PCM treatment following exercise of mechanical nature, these findings must be investigated in an EIMD model induced primarily by metabolic stress. Especially since it has been proposed that CWI may be more effective for recovery from metabolically stressful exercise, as opposed to mechanically stressful exercise (Ihsan et al., 2016; Leeder et al., 2012). Thus, chapter 5 will administer 3 hours of PCM cooling to both quadriceps in the treatment condition following a marathon run.

Interestingly, the findings of the present chapter indicate that local PCM application might have acted systemically in the contralateral limb since the soreness and strength loss reported for the direct cooling and indirect cooling conditions were not different. Since the results of this chapter indicate that direct cooling one leg might have influenced the recovery of strength and soreness of the contralateral leg (indirect cooling), chapter 6 will aim to establish the degree of temperature reduction achieved in the muscle and within the core of the body from 3 hours of PCM cooling, in order to ascertain whether the response observed in the limb contralateral to the direct cooling was indeed a true systemic effect.

**5.0 THE EFFICACY OF
PROLONGED PHASE CHANGE
MATERIAL COOLING FOR
ENHANCING RECOVERY
FOLLOWING MARATHON**

5.1 Introduction

Although muscle damage can be induced by either mechanical or metabolic stress (Tee et al., 2007), the events occurring during exercise with a large metabolic component such as a marathon differ from those occurring from exercise encompassing primarily mechanical components such as the isolated eccentric exercise performed in chapter 4. The processes occurring during and following a marathon are multifactorial relating to not only peripheral factors within the skeletal muscles associated with EIMD, but also an increase in the magnitude of thermal load (Deschenes et al., 1998; Mortensen et al., 2008), as well as central nervous system fatigue (Petersen et al., 2007). Functional performance, strength loss, soreness, muscle damage, inflammation and oxidative stress have all been reported following marathon length runs (Davies & Thompson, 1986; Kratz et al., 2002; Maron et al., 1977; Millet et al., 2002, 2003; Nicol et al., 1991; Petersen et al., 2007; Place et al., 2004; Starkie et al., 2001; Suzuki et al., 2003). These symptoms can persist for extended periods following a marathon even in experienced runners (Maron et al., 1977; Petersen et al., 2007). Indeed, following a marathon, the repair of focal muscle fibre damage has been reported to take up to 8 weeks (Warhol et al., 1985). As a result, marathon runners have turned to a range of recovery strategies to mitigate their symptoms after training and competitive runs in an attempt to speed up the regenerative process. Some popular strategies include cryotherapy (Wilson et al., 2018), compression garments (Hill et al., 2014a), or nutritional interventions (Clifford et al., 2016; Howatson et al., 2010).

Cryotherapy interventions, used to mitigate symptoms of EIMD and to accelerate recovery, have become increasingly popular amongst athletes. One of the most popular cryotherapy modalities among athletes is CWI (see section 2.2.5.3). However, it has recently been suggested that the efficacy of CWI for attenuating the effects associated with EIMD might be dependent on the mode of exercise utilised. In a review of the literature, CWI was found to be more effective in ameliorating effects of EIMD induced by metabolically stressful whole body exercise, such as prolonged running, than following mechanically stressful single joint exercise, such as high intensity isolated eccentric contractions (Ihsan et al., 2016). Similarly, in a meta-analysis Leeder et al. (2011) determined that CWI was effective in alleviating soreness at 24 and 48 hours after high intensity whole body exercise but only moderately effective at 48 hours following isolated eccentric exercise. Thus, CWI may be more successful in accelerating recovery following a marathon than following isolated eccentric exercise.

Unfortunately, the only study to have previously investigated the effectiveness of cryotherapy for recovery from a marathon concluded that cryotherapy was no more effective than a placebo intervention at improving functional recovery or perceptions of training stress (Wilson et al., 2018). The authors administered a CWI protocol of 8°C for 10 minutes immediately following completion of the marathon run. It is likely that this duration of immersion did not sufficiently reduce muscle temperature for a period long enough to diminish the extensive secondary phase of muscle damage that occurs following a marathon run. Indeed, in studies administering one continuous immersion, the average immersion duration was 12.6 minutes at a mean temperature of 13°C (Bleakley et al., 2010; Versey, Halson, & Dawson, 2013). Although an optimal dose for cryotherapy application has yet to be established, Vromans, Thorpe, Viroux, & Tiemessen (2019) have recently suggested that the most optimal dose-response relationship for CWI protocols using full-body immersion occurs when a minimum CWI dose of 1.1 is applied. This corresponds with an immersion duration of 11 minutes in a water temperature of 10°C. Unfortunately their study did not account for any differences in the elevated thermal load that occurs from prolonged endurance exercise, as opposed to the relatively smaller overall thermal load occurring during resistance exercise. Therefore, it seems counterintuitive to expect a recovery intervention, administered for only 13 minutes following a marathon run lasting on average 4 hours, to have significant effects on the signs and symptoms associated with EIMD resulting from an exercise of such magnitude. Increasing the cryotherapy dose following endurance exercise, to account for elevations in muscle and core temperature, might be necessary for the cryotherapy modality to elicit a similar response following endurance exercise than following resistance exercise.

A longer duration of cooling can be safely achieved by using PCM packs that freeze at 15°C (see section 2.2.5.4). The efficacy of 6 hours of PCM cooling for recovery of strength and soreness following eccentric exercise was demonstrated in chapter 4. Whilst 3 hours of PCM cooling has also been shown to accelerate recovery of strength (Brownstein et al., 2019; Clifford et al., 2018) and soreness (Clifford et al., 2018) following soccer match play. Although the previous chapter demonstrated that PCM was capable of accelerating recovery following isolated eccentric exercise, it remains unknown whether PCM will do the same following exercise with a large metabolic component. As the overall aim of this thesis is to determine the efficacy of prolonged PCM cooling for recovery from exercise, this chapter examined the effects of prolonged PCM cooling on recovery of strength, soreness, functional performance, and blood markers of muscle damage (CK) and inflammation (hsCRP) following the

completion of a marathon run. It was hypothesized that 3 hours of prolonged PCM cooling would accelerate recovery of strength loss, soreness, CMJ height, and blood markers of muscle damage and inflammation on the days following the marathon run. Therefore, this chapter will contribute to the overall aims of this thesis by providing insight into the efficacy of prolonged PCM cooling following exercise with a major metabolic component. The results of this chapter will be contrasted and compared with the results of the previous chapter to determine whether PCM cooling is similarly effective for accelerating recovery following exercise of both metabolic and mechanical nature.

5.2 Methods

5.2.1 Participants

A power calculation was conducted based on the available literature on marathon running to determine an adequate sample size for this study. Estimates were made of the expected change, and the inter-subject variation in change for each marker of muscle damage (Howatson et al., 2010; Duthie et al., 1990; Kratz et al., 2002; Suzuki et al., 2003; Smith et al., 2004). Assuming that the control group would have the expected responses, estimates were made on how much lower that response would need to be in the PCM treatment group. Thus, it was estimated that there would be 80% power to detect a 10% difference in strength loss between treatments (SD: 8.0%, based on % change from baseline data for muscle damage) at an alpha level of 0.05 using a one-sided t-test with a total of 15 participants per group.

As such, thirty healthy volunteers, 11 male and 19 female, participated in the study (mean \pm SD; age, 34 \pm 8 years; height, 169.4 \pm 10.7 cm; body mass, 68.1 \pm 12.9 kg). Participants were runners with varying degrees of marathon experience (number of previous marathons: 5 \pm 6) whose expected completion times were 4:10 \pm 0:42 hours. Female participants verbally confirmed that they were premenopausal. In the 5 days prior to the marathon run, and for the duration of the study, participants adhered to the controls described in section 3.4. The institutional research ethics committee, in line with the Declaration of Helsinki, approved all procedures.

5.2.2 Experimental Design

Participants reported to the laboratory for a total of four days. On the first day of data collection (baseline testing) participants completed an elite performance readiness questionnaire, before being randomly assigned into either the PCM treatment group

(n = 15; 6 male, 9 female) or the control group (n = 15; 5 male, 10 female). Participants were paired and equally assigned to either group based upon both predicted finish time and sex in a pseudorandomised fashion, in an attempt to account for possible sex differences in response to marathon running. Participants were then familiarised with all of the testing procedures prior to baseline data collection of all dependent variables. Measurements of all outcome variables were also recorded one, two and three days following completion of the marathon run. The following marathons were completed by study participants: New York City (n = 26), Philadelphia (n = 1), Brooklyn (n = 1), London (n = 1), and Cape Cod (n = 1). All marathons were road routes. The terrains for these marathons were similar with the courses being 38-43% downhill and 37-41% uphill, with a total elevation of 894.5 ± 281.4 feet. Participants ran at a self-selected pace, and were allowed to consume fluids, electrolytes and/or food ad libitum during the marathon but were asked to avoid consuming any supplements containing BCAAs, protein, antioxidants or caffeine.

5.2.3 Phase Change Material Application

Participants in the PCM treatment group wore the frozen (15°C) PCM over the quadriceps of both legs for a total of 3 hours. Participants receiving PCM treatment began the intervention within $1:20 \pm 0:34$ hours of finishing the marathon. For complete PCM application procedures, please refer to section 3.5.

5.2.4 Strength Assessment

Strength was tested at 80° knee flexion. For complete strength testing procedures, please refer to section 3.8.

5.2.5 Soreness Assessment

For procedures to assess muscle soreness, please refer to section 3.10.

5.2.6 Countermovement Jump

Countermovement jumps were performed using the Optojump optoelectric system (Bolzano, Italy). This optical measurement system is considered a valid and reliable tool to evaluate CMJ performance (Glatthorn et al., 2011). A reliability trial conducted before data collection revealed that the inter-day CV for this protocol was 3.6%. Prior to data collection, participants were given an unlimited number of trial sessions to be performed at ~50% maximal effort, until their jumping technique was performed correctly according to the following instructions. Participants started the movement

upright with their hands fixed to their hips and after a verbal cue, descended into a self-selected squat position prior to performing a maximal effort vertical jump. Participants were instructed to maintain no bend in their knees at the peak of the jump. If a participant bent their knees at the peak of the jump, the jump did not count towards a maximal effort. Once the participant's technique was correct, participants performed 3 maximal efforts, separated by approximately 60 seconds of standing recovery; the mean of the 3 jumps was used for analysis. Jump heights were recorded in centimetres. CMJs were performed prior to strength testing.

5.2.7 Blood Sampling and Analysis

For blood sampling procedures and analysis, please refer to section 3.11.

5.2.8 Statistical Analysis

All data are presented as mean \pm SD. The effect of PCM cooling or control on strength loss, soreness, CMJ height, CK, and hsCRP on the three days following the marathon was assessed using 2 \times 4 treatment by time mixed-model ANOVA. The two levels for the treatment factor were PCM cooling and control. The four levels for the time factor were baseline and day 1, day 2, day 3, after the marathon. Baseline values were compared between treatment groups using independent t-test. There were technical issues with measuring CMJ for 7 participants throughout the duration of the study. Thus, only 12 and 11 participants in the control and PCM treatment groups, respectively, were included in the treatment by time analysis of the CMJ data.

Normality of all data sets was examined using the Shapiro-Wilk test. Mauchly's test was used to assess assumptions of sphericity and, where necessary, Greenhouse–Geisser corrections were used. Differences between treatments at any particular time interval were assessed with independent t-tests using Bonferroni corrections for planned pairwise comparisons. Where appropriate, Cohen's d ES statistics were calculated to provide magnitude of effects; with the magnitude of effects considered either small (0.20-0.49), medium (0.50-0.79), and large (>0.80). The upper and lower limit of 95% confidence intervals (CI) for least significant difference are reported where relevant. Statistical analyses were performed using SPSS v.21 (IBM, Armonk, NY, USA) and a P-value of less than 0.05 was considered statistically significant.

5.3 Results

A summary of participant characteristics and completion times are presented in Table 3.

Table 3: Participant characteristics and marathon completion times. Values are mean \pm SD.

Treatment	<i>n</i>	Age (years)	Height (cm)	Weight (kg)	# Previous Marathons	Expected Finish Time	Actual Finish Time
PCM	15	36\pm8.1	170.9\pm10.0	67.1\pm11.5	3\pm6	4:21\pm0:42	4:23\pm0:53
Female	9	35 \pm 8.6	164.6 \pm 6.7	60.5 \pm 6.7	4 \pm 6	4:16 \pm 0:43	4:29 \pm 0:51
Male	6	37 \pm 7.7	180.3 \pm 5.8	77.1 \pm 10.1	10 \pm 7	3:34 \pm 0:22	3:40 \pm 0:20
Control	15	33\pm8.8	167.9\pm11.5	69.1\pm14.4	6\pm7	3:59\pm0:40	4:11\pm0:48
Female	10	31 \pm 8.2	162.3 \pm 7.4	62.8 \pm 12.3	3 \pm 7	4:26 \pm 0:46	4:29 \pm 0:58
Male	5	36.6 \pm 9.3	179.0 \pm 10.4	81.7 \pm 9.5	2 \pm 2	4:11 \pm 0:36	4:13 \pm 0:47
Total	30	34\pm8.4	169.4\pm10.7	68.1\pm12.9	5\pm6	4:10\pm0:42	4:17\pm0:50
<i>Between group comparisons</i>		P = 0.338	P = 0.454	P = 0.689	P = 0.143	P = 0.169	P = 0.543

Note: Finish times are reported as h:mm.

5.3.1 Strength

At baseline average knee extension peak torque was not different between groups ($P = 0.771$; PCM treatment: 157.9 ± 36.6 Nm, control: 154.0 ± 35.3 Nm). Average quadriceps strength expressed as a percentage change from baseline for the 3 days following the marathon showed a main effect of time ($F = 15.0$, $P < 0.0001$, $ES = 1.46$; Figure 11). However, overall recovery of MIVC was independent of treatment with no group ($P = 0.535$) or interaction effect observed ($P = 0.828$), indicating that the recovery of strength over the 3 days following the marathon was comparable in both treatment and control conditions. For illustrative purposes, torque data for each day of data collection of each bout are presented in Table 4 to demonstrate the return of function following the exercise protocol.

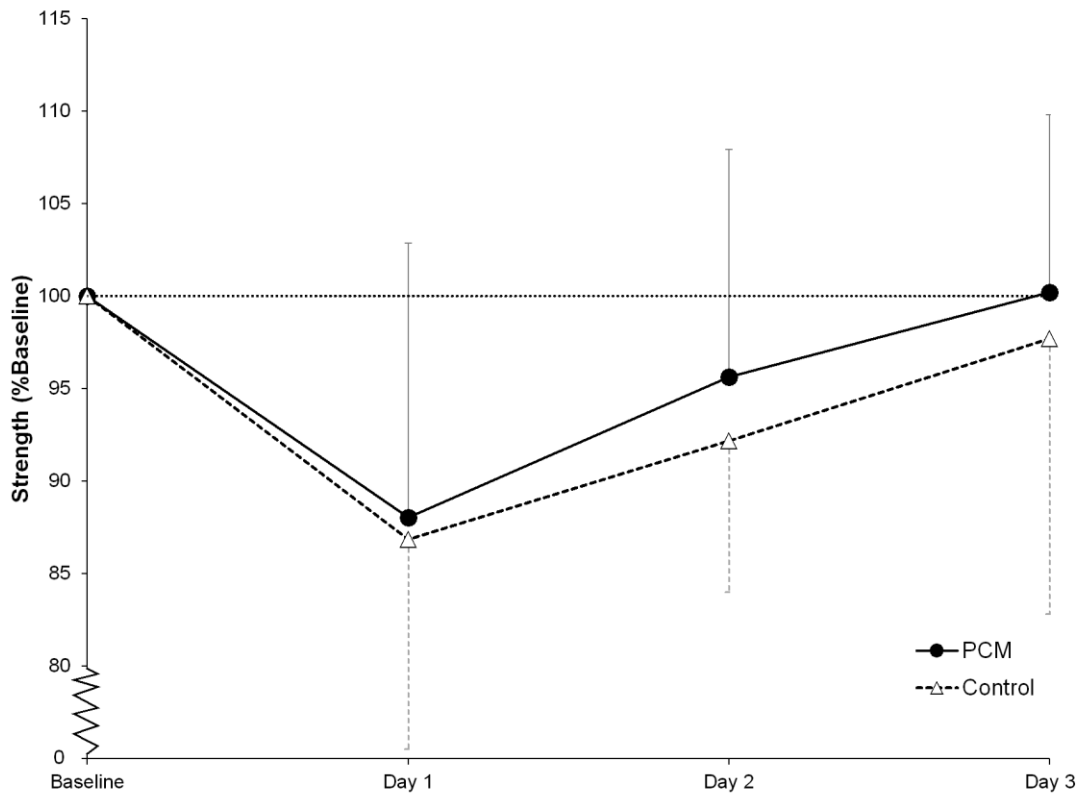


Figure 11: Isometric strength loss of the quadriceps (presented as a percentage change from baseline strength loss) for the PCM treatment and control groups before and on the three days following the marathon. Values are mean \pm SD. Strength was reduced over the 4 days after the marathon ($P < 0.0001$), with no difference between groups.

Table 4: Isometric strength (MIVC) of the quadriceps for the PCM treatment and control conditions reported as net torque values (Nm) on each day after the marathon.

	PCM (Nm)	Control (Nm)
Baseline	156 \pm 34	154 \pm 34
Day 1	137 \pm 32	133 \pm 32
Day 2	149 \pm 33	141 \pm 33
Day 3	156 \pm 35	148 \pm 35
<i>Time Effect</i>	P = 0.002	P < 0.0001

Note: Values are mean \pm SD.

5.3.2 Soreness

At baseline, quadriceps soreness was not different between groups ($P = 0.254$; PCM treatment: 0.2 ± 0.8 , control: 0.0 ± 0.0 , VAS 0-10). Following the marathon, perceptions of soreness increased over time (Figure 12; $F = 76.4$, $P < 0.0001$, $ES = 3.31$). Overall recovery of soreness was independent of treatment with no treatment ($P = 0.264$) or interaction effect observed ($P = 0.195$). Interestingly, peak soreness was inversely correlated with number of prior marathons ($P = 0.005$, $r = -0.497$), but this effect was not different across treatments ($P = 0.544$).

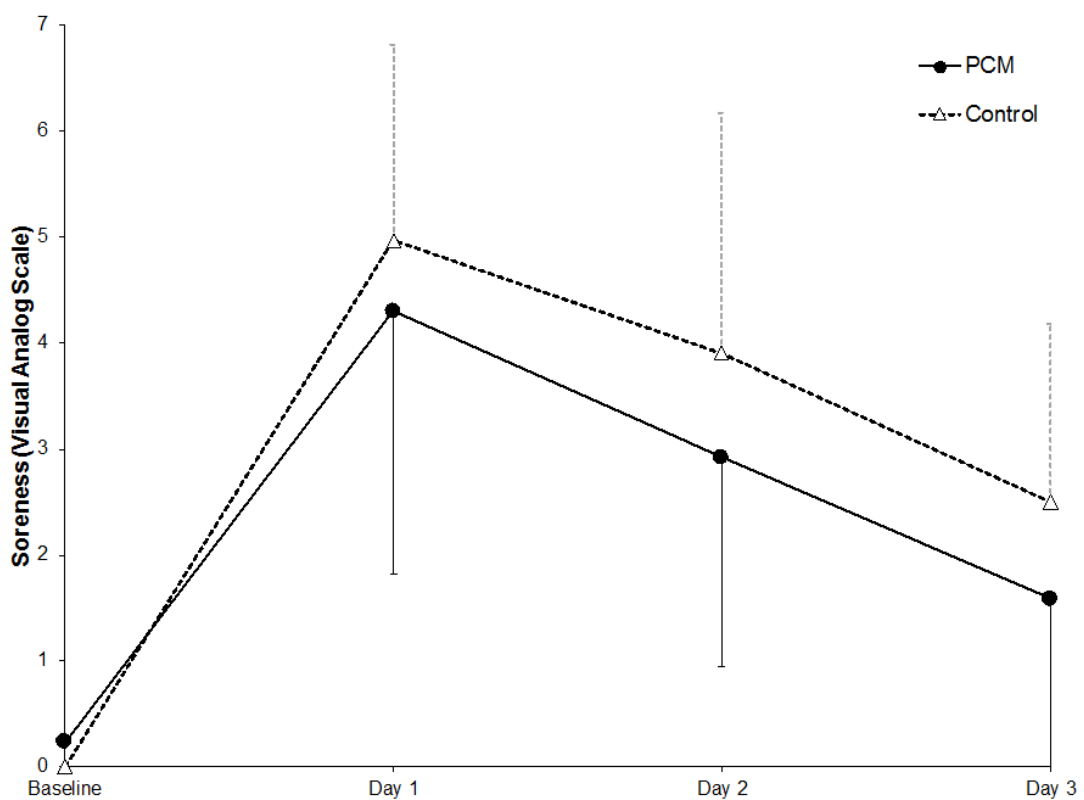


Figure 12: Subjective reports of quadriceps soreness on a 0-10 scale (0 = no discomfort, 10 = too painful to squat to 90°) for the PCM treatment and control groups before and for three days following the marathon. Values are mean \pm SD. Soreness following the marathon was increased in both groups ($P < 0.0001$).

5.3.3 Countermovement Jump Height

Jump height was not different between groups at baseline ($P = 0.764$; PCM treatment: 21.2 ± 4.4 cm, control: 21.9 ± 5.5 cm). Jump height decreased following the marathon (Figure 13; $F = 15.9$, $P < 0.0001$, $ES = 1.74$). There was no difference between treatments in the recovery of CMJ, with no treatment ($P = 0.273$) or interaction effect observed ($P = 0.198$).

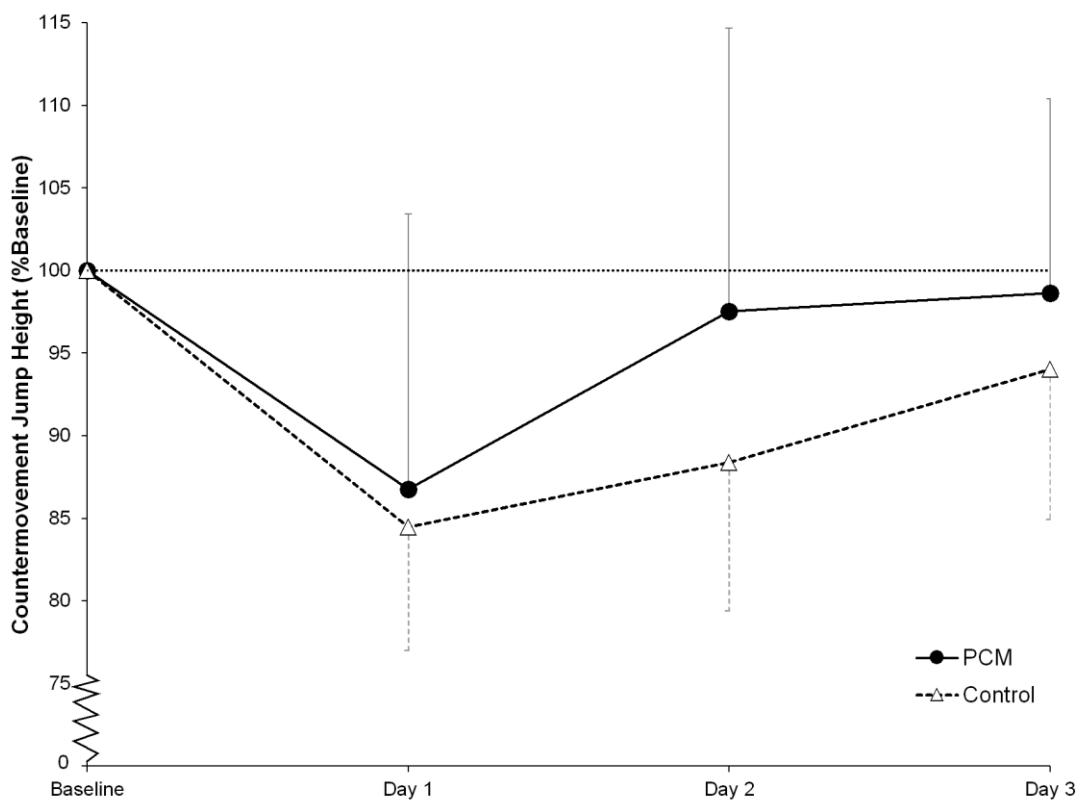


Figure 13: Percentage change from baseline in countermovement jump (CMJ) height for the PCM treatment and control groups before and for three days following the marathon. Values are mean \pm SD. Following the marathon, CMJ height was impaired in both groups ($P < 0.0001$), but was not different between treatments.

5.3.4 Blood Markers

At baseline, CK values were not different between groups ($P = 0.224$; PCM treatment: $119.0 \pm 71.0 \text{ U}\cdot\text{L}^{-1}$, control: $169.2 \pm 139.0 \text{ U}\cdot\text{L}^{-1}$). At baseline, CRP values were not different between groups ($P = 0.267$; PCM treatment: $1.03 \pm 0.82 \text{ ug/ml}$, control: $0.77 \pm 0.34 \text{ ug/ml}$). Following the marathon, CK and hsCRP increased over time in both groups (Table 5), with no difference between the PCM treatment and control conditions (CK: $P = 0.623$; hsCRP: $P = 0.655$) and no interaction effects (CK: $P = 0.309$; hsCRP: $P = 0.412$).

Table 5: Response of indices of muscle damage (serum Creatine Kinase; CK) and inflammation (high sensitivity C-Reactive Protein; hsCRP) in the blood before (baseline) and for 3 days (Day 1, 2, and 3) after the marathon run in the PCM and Control groups. Values are mean \pm SD.

	CK ($\text{U}\cdot\text{L}^{-1}$)		hcCRP (ug/ml)	
	PCM	Control	PCM	Control
Baseline	119 \pm 70 (2.00 \pm 0.28)	169 \pm 138 (2.11 \pm 0.31)	1.03 \pm 0.82 (-0.07 \pm 0.26)	0.77 \pm 0.34 (-0.15 \pm 0.17)
Day 1	893 \pm 471 (2.87 \pm 0.30)	841 \pm 372 (2.87 \pm 0.24)	7.72 \pm 5.28 (0.79 \pm 0.31)	7.38 \pm 4.56 (0.80 \pm 0.27)
Day 2	419 \pm 289 (2.51 \pm 0.35)	547 \pm 363 (2.64 \pm 0.31)	3.63 \pm 1.89 (0.51 \pm 0.22)	4.27 \pm 2.29 (0.56 \pm 0.28)
Day 3	328 \pm 193 (2.42 \pm 0.32)	378 \pm 416 (2.36 \pm 0.47)	2.26 \pm 1.12 (0.30 \pm 0.24)	4.23 \pm 5.80 (0.43 \pm 0.39)
<i>Time Effect</i>	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Note: Values are presented as absolute value (log transformed value).

5.4 Discussion

This study investigated whether administering 3 hours of PCM cooling would accelerate recovery following a marathon run. The marathon resulted in reduced muscle function, increased perceptions of soreness, and increased concentrations in the blood markers of muscle damage (CK) and inflammation (hsCRP). Contrary to the hypothesis, and unlike the findings of the previous chapter, there was no

difference in the rate of recovery between the PCM treatment and control groups. The results of the present chapter indicate that prolonged PCM cooling was not an effective recovery strategy when administered after running a marathon.

The findings of the present study are in agreement with the only other previous study to have investigated the effectiveness of cryotherapy for recovery from a marathon (Wilson et al., 2018). Wilson et al. (2018) demonstrated that compared with control neither CWI nor WBC accelerated recovery of strength loss, soreness, muscle function, and blood markers of muscle damage and inflammation after a marathon run. Importantly the marathon run in their study did not elicit any strength loss, making it difficult to determine whether the cryotherapy interventions were effective. Furthermore, Wilson et al. (2018) reported that their participants were trained endurance runners and that the course of their marathon consisted of an outdoor route comprised of predominantly grass and unpaved footpaths, with some short concrete sections run at a self-selected pace. Since muscle soreness was largely absent by the second day following the marathon, coupled with no effect from the marathon on strength loss, their findings indicate that the marathon run was not strenuous enough to elicit a damage response. Comparably, the participants in the present study were less trained, had longer marathon completion times (4:17 vs 3:48 hours), elevated soreness on the second day that had not recovered in either group by the third day following the marathon, and increased blood markers of muscle damage and inflammation. Additionally, participants in the present study experienced a 12% and 13% reduction in MIVC one day following the marathon in the PCM and control groups respectively, with strength returning to baseline in the PCM treatment group by day 2 but not until day 3 in the control group. Hence, the damage response in the present study was greater than that reported by Wilson et al. (2018). The 13% strength loss and 5 out of 10 pain on day 1 in the control group of the present study are comparable to values reported by Howatson et al. (2010; 18% strength loss and 4 out of 10 pain on day 1). Thus, in the present study, there was sufficient damage from the marathon to detect a benefit from the PCM cooling intervention.

There was also no benefit from PCM treatment, compared to control, for accelerating CMJ height following the marathon. However, it is notable that, compared to baseline, the PCM treatment group did not exhibit compromised jump height on any of the three days following the marathon (Day 1: 87% of baseline, Day 2: 98%, Day 3: 99%). On the other hand, jump height was impaired for two days after the marathon in the control group (Day 1: 84%, Day 2: 88%). This finding has some value considering that CMJ may be a better indicator of overall muscle function than isolated strength

measures. Significant decrements in CMJ height have previously been reported for 2 days following marathon running (Petersen et al., 2007). One previous study measured CMJ height following a marathon and showed a 10% and 5% reduction in jump height on day 1 and 2, respectively; with no benefit from a nutritional intervention in accelerating recovery of CMJ height (Clifford et al., 2016). Comparably, only one previous study has examined the effects of CWI on CMJ height following a half-marathon, reporting minimal impairment of CMJ height and subsequently no effect of CWI in accelerating recovery of CMJ height after the half-marathon (4% reduction; Wiewelhove et al., 2018).

There are limited studies investigating the potential effectiveness of recovery strategies in road running. Further, few of these studies have successfully implemented a recovery intervention to ameliorate any of the signs and symptoms following marathon running (Clifford et al., 2016; Hill et al., 2014a; Howatson et al., 2010). One study successfully administered a lower limb compression garment which reduced perceived muscle soreness one day after the marathon but did not affect strength (Hill et al., 2014a). One study implemented a pre- and post-marathon nutritional intervention which successfully accelerated recovery of strength but did not affect soreness (Howatson et al., 2010). In the third study, there was insufficient muscle damage from the marathon to determine an effect of the nutritional intervention (Clifford et al., 2016). Interestingly, the nutritional intervention investigated by Clifford et al. (2016) was provided only on the 3 days after the marathon, while runners in the treatment group of Howatson's (2010) study received the nutritional intervention for 5 days prior to the marathon, as well as on the 2 days after the marathon. Hence it was possible that consuming a nutritional intervention prior to the exercise stimulus acted systemically as a pre-recovery modality to mitigate some part of the metabolic response that occurred during a marathon run. Indeed, the inflammatory response was blunted in the runners receiving a nutritional intervention prior to running a marathon (Howatson et al., 2010), and this may have limited the subsequent exacerbation of secondary damage. Since administering cryotherapy prior to exercise is generally contraindicated, the PCM packs were not implemented as a pre-recovery strategy in the present study, and ultimately the inflammatory response was not reduced by the PCM treatment.

The previous chapter indicated that PCM cooling successfully accelerated recovery following eccentric exercise encompassing a mechanical component of muscle damage. Unlike the findings of the previous chapter, this study indicated that PCM did not accelerate recovery following a marathon, which involves a large metabolic

component. It is possible that the cooling effect from the PCM packs applied locally to the quadriceps was not large enough to act systemically on a damage response occurring across several muscle groups. This is especially likely when considering that endurance type protocols typically produce a greater level of systemic hyperthermia and different metabolic perturbations, compared with isolated exercise such as resistance exercise (Deschenes et al., 1998; Mortensen et al., 2008). Therefore a larger magnitude of cooling would be necessary to elicit the same response following a marathon than following isolated eccentric exercise. Alternatively, the PCM cooling treatment in the present study might have been administered too late into the muscle damage cycle, once secondary damage had already occurred. Lastly, it is possible that the mechanisms through which cryotherapy accelerates recovery are effective following exercise involving primarily mechanical muscle damage, but not following exercise involving prolonged exposure to metabolic stress. A few theories exist to explain the difference in response to metabolic and mechanical stress (Tee et al., 2007). One of the more pronounced features of endurance exercise is an elevation in systemic inflammation (see section 2.1.2.2; Shin & Lee, 2013; Suzuki et al., 2003), which is independent of the training status of the runner (Santos et al., 2016). Indeed, the marathon run markedly elevated hsCRP in the present study, but PCM did not effectively accelerate the recovery of this response. However, overall there is insufficient data to support the theory that cryotherapy reduces the post-exercise inflammatory response. Ultimately it is most likely that magnitude of the cryotherapy dose was insufficient in the present study.

It is important to acknowledge the other limitations of this study. Due to the nature of marathon environments with large numbers of runners, retrieving one's belongings following the race was delayed, as a result the PCM were not applied until on average 80 minutes following completion of the marathon. Adding to this delay in PCM application, during such a long-distance run, muscle damage is likely induced before the marathon run is concluded; thus, an overlap exists between the individual exercising and muscle damage occurring. This overlap was likely significantly smaller in previous studies showing a beneficial effect from PCM where the exercise took between 40 minutes (chapter 4) to 90 minutes (Brownstein et al., 2019; Clifford et al., 2018) to complete and PCM were applied within 10 (chapter 4), 30 (Clifford et al., 2018), and 45 minutes (Brownstein et al., 2019) of exercise cessation. Animal models have previously shown that a window of opportunity for intervention with cryotherapy lies within the first 30 minutes after injury (Merrick & McBrier, 2010). Thus, in the present study, a combination of exercise that had high metabolic stress for a

prolonged duration (on average exceeded four hours) and a delay in the application of PCM cooling might have limited the potential of the intervention to accelerate recovery. In an ideal scenario, PCM cooling would be administered as soon as possible upon cessation of exercise. This timing was likely more successfully achieved in chapter 4 following 40 minutes of isolated eccentric contractions or a 90-minute soccer game (Brownstein et al., 2019; Clifford et al., 2018).

5.5 Conclusion

The present study expands upon the findings of chapter 4, as well as previous studies administering PCM for 3 and 6 hours following isolated eccentric knee extensions and a soccer match attenuated strength loss (chapter 4 and 7; Brownstein et al., 2019 and Clifford et al., 2018, respectively) and/or soreness (chapter 4 and 7; Clifford et al., 2018, respectively). Chapter 4 indicated that PCM can successfully accelerate recovery following EIMD comprised of a large mechanical stress component. However, the present study demonstrated that prolonged PCM cooling was not effective in accelerating recovery following exercise encompassing a large metabolic stress component. Therefore, the lack of effect in the present study might be due to the mechanisms of PCM acting differently following high-intensity exercise with a significant eccentric component than following a marathon. However, it remains unknown whether the physiological responses to prolonged PCM cooling following exercise of primarily metabolic or mechanical nature are different. Based on these data, local PCM cooling might not provide a sufficient cooling stimulus to elicit a systemic response in order to accelerate recovery following prolonged endurance exercise, although this should be confirmed with a tightly controlled experimental paradigm.

5.6 Perspectives

The overall aim of this thesis is to establish the efficacy of prolonged cooling using PCM for attenuating muscle damage and accelerating recovery from exercise. In the previous chapter, PCM cooling hastened the recovery of strength and soreness following eccentric exercise of the quadriceps. Subsequently, it was expected that PCM cooling would also accelerate recovery following a marathon. Especially since marathon running is associated with a large muscle damage response and inflammatory response. However, the results of the present chapter demonstrated that prolonged PCM cooling was not effective for accelerating recovery of muscle function, perceptions of soreness, and blood markers of muscle damage and

inflammation following marathon running. Although there was no difference between the PCM treatment and control groups in any of these variables, strength was only significantly reduced below baseline on day 1 and returned to baseline by day 3 in the PCM treatment group, while in the control group strength was significantly reduced below baseline on days 1 and 2 and did not and return to baseline by day 3. Comparably, CMJ height was not significantly reduced below baseline on any of the days following the marathon in the PCM treatment group but remained compromised on days 1 and 2 in the control group. Although not significantly different between treatments, upon visual inspection soreness, muscle damage and inflammation (hsCRP) were lower in the PCM treatment group than in control. Although the results from this study do not support the use of PCM to alleviate muscle damage and accelerate recovery following a marathon run, it is possible that a larger cooling stimulus such as PCM applied to the entire leg and not just the quadriceps might have elicited a more favourable result.

Apart from the difference in duration of PCM cooling in the previous chapter (6 hours) and the present chapter (3 hours), the major factor that might explain the discrepancy in the results between the two studies is that the muscle damage models were different. The study in the previous chapter employed isolated eccentric exercise to induce muscle damage. In that instance, muscle damage was induced solely through direct mechanical disruption. On the contrary, although marathon running involves a large number of stretch-shortening cycles, structural damage occurs due to the continued demand in the face of substrate depletion. Furthermore, the muscle damage in chapter 4 was localised to one muscle group (quadriceps), and it was the quadriceps that were directly cooled following the eccentric exercise. In the present study, during the marathon run, muscle damage was not localised to a single muscle group and occurred in more than just the quadriceps, but only the quadriceps were cooled. Thus, the localised cooling stimulus was likely insufficient to elicit any beneficial effects to the peripheral musculature that was also likely experiencing a damage response and was clearly experiencing a systemic inflammatory response (see hsCRP data in Table 5).

The results from this chapter compared with those of the previous chapter, that PCM cooling was not effective following a marathon but accelerated recovery following eccentric exercise, address the overall aims of this thesis by indicating that prolonged PCM cooling can be effectively utilised as a recovery modality following exercise encompassing mechanical stress but not metabolic stress. The following chapter of

this thesis will attempt to isolate the effects of 3 hours of PCM cooling at the quadriceps, in non-exercising rested individuals, in order to determine the physiological effects of cooling at the quadriceps muscle and the core. The effect of local PCM cooling on core temperature will answer some of the questions arising from this chapter and the previous chapter, because an effect on core temperature will indicate that PCM cooling acts both locally and systemically. This result would explain the systemic effect evident in chapter 4 and would further indicate that the local PCM cooling following the marathon run in the present chapter was too little of a cooling stimulus to elicit a systemic effect on recovery. Further, although the PCM intervention was not successful in accelerating recovery in this chapter, it was evident that the marathon induced elevations in blood markers of muscle damage and inflammation over the 3 days after the run. However, blood markers of muscle damage or inflammation were not measured following the mechanically induced muscle damage in the previous chapter. Therefore, in order to compare the effects of PCM cooling on muscle damage and inflammation occurring from metabolic stress vs mechanical stress, chapter 7 will aim to quantify the degree of muscle damage and inflammation evident in the blood following isolated eccentric exercise.

6.0 EXPLORING THE EFFICACY OF A SAFE CRYOTHERAPY ALTERNATIVE: PHYSIOLOGICAL TEMPERATURE CHANGES FROM COLD WATER IMMERSION vs PROLONGED PHASE CHANGE MATERIAL COOLING

This chapter has been accepted for publication:

Kwiecien SY, McHugh MP, Goodall S, Hicks KM, Hunter AM, Howatson G. (2019). Exploring the efficacy of a safe cryotherapy alternative: physiological temperature changes from cold-water immersion versus prolonged cooling of phase-change material. *Int J Sports Physiol Perform.* 14(9):1288-1296.

6.1 Introduction

The previous chapters established the efficacy of prolonged cooling using PCM for accelerating recovery following mechanically stressful eccentric exercise (chapter 4), but not following marathon running which is an exercise with a significant metabolically stressful component (chapter 5). Further, PCM cooling has also been used effectively for accelerating recovery following match play in professional soccer players (Brownstein et al., 2019; Clifford et al., 2018), which encompasses components of both mechanical and metabolic stress. Therefore, it would appear that either the mechanisms through which PCM accelerated recovery in the previous two chapters varied or that the magnitude of the cooling dose was sufficient following the mechanically stressful exercise in chapter 4 but not following the metabolically stressful exercise in chapter 5.

Many of the physiological changes suspected to be integral to the therapeutic effects of cryotherapy are thought to be temperature-dependent (White & Wells, 2013) and dependant on the intensity of the cold stimulus (Crowe et al., 2007) and how it affects the body (García-Manso et al., 2011). The thermal and cardiovascular responses to CWI have recently been reviewed by Stephens (2017). Briefly, recovery following exercise is purported to be enhanced by CWI primarily due to its ability to reduce muscle temperature (Mawhinney et al., 2013; 2017; Roberts et al., 2014; 2015); with the skin temperature playing a relatively minor role (Vargas et al., 2018). Core temperature can also be reduced by CWI if the immersion duration is long enough to sufficiently elicit a systemic response (Costello et al., 2012a; Gregson et al., 2011; Stephens et al., 2018). Additionally, CWI has also been demonstrated to restore the cardiovascular response following exercise as measured using indices of heart rate variability (HRV; Al Haddad et al., 2010; Almeida et al., 2016; Bastos et al., 2012).

Since typical CWI protocols involve a single post-exercise treatment for 10-15 minutes in water temperatures between 10-15°C (Bleakley et al., 2012; Leeder et al., 2012), limited effectiveness might be a result of inadequate treatment duration, temperature, or both. Although lower immersion temperatures might be more effective in the treatment of EIMD by virtue of greater reductions in muscle temperature (Mawhinney et al., 2013), protocols of excessive cooling capacity (colder temperatures) may result in sympathetic activation (Datta & Tipton, 2006), or may exacerbate the pro-inflammatory response (White et al., 2014). Concurrently, prolonging the treatment duration of cryotherapy at very low temperatures puts the superficial tissues at risk of developing cold-related injury (Enwemeka et al., 2002;

Nadler et al., 2003; Swenson et al., 1996), may lead to excessive thermal discomfort and, if prolonged, is not well tolerated (Howatson et al., 2016). In practice, if the goal is to increase the period for which the aforementioned physiological effects are maintained (decreased muscle and core temperature), repeat treatments may be necessary but are impractical and present logistical challenges. A longer duration of targeted post-exercise cooling can be achieved using PCM; however, the cooling efficacy of PCM was not investigated in the previous chapters.

Prolonging the duration of tissue cooling has previously been suggested as a critical component in reducing the secondary muscle damage response (Merrick et al., 1999; Swenson et al., 1996) and mitigating the overall extent of tissue damage (Bleakley et al., 2012; Enwemeka et al., 2002; Ihsan et al., 2013; Knight, 1995). Merrick et al. (1999) were the first to provide empirical evidence that a prolonged dose of cryotherapy was effective in retarding secondary injury. The authors concluded that the 5 hours of continuous cold application maximized the injury-reducing effect of cryotherapy and suggested that the prolonged duration of application may be advantageous. Bleakley et al. (2012) suggested that prolonging the cooling duration would be necessary in humans in order to achieve similar magnitudes of intramuscular cooling evident in animal models. Enwemeka et al. (2002) demonstrated that 20 minutes of cooling was insufficient to reduce intramuscular temperature at the deep tissue. Similarly, Ihsan et al. (2013) concluded that the magnitude of reduction in muscle perfusion and metabolic activity from 15 minutes of CWI was insufficient to promote noticeable physiologic changes associated with minimizing muscle damage or preserving muscle function. With these recommendations in mind, it is logical that prolonging the dose of cryotherapy may overcome some of the aforementioned limitations.

The overall aim of this thesis is to assess the efficacy of prolonged PCM cooling as a recovery modality following exercise. As was evident in chapter 4, 6 hours of PCM cooling accelerated indices of recovery following eccentric exercise; while 3 hours of cooling in chapter 5 were unsuccessful in enhancing recovery following marathon running. Thus, it was possible that the 6 hours of PCM cooling in chapter 4, but not the 3 hours of cooling in chapter 5, attenuated some mechanism occurring during the stage of secondary damage following isolated eccentric exercise but not following a marathon run encompassing prolonged metabolic stress. However, in the previous two chapters it was unknown to what extent prolonged PCM cooling might have exerted effects on body temperature and cardiovascular measures, similar to those

occurring from CWI. Additionally, it was unknown whether muscle and core temperatures remained reduced throughout the duration of PCM treatment. For this reason, this chapter aimed to understand the physiological effects (muscle, core, skin temperature and HRV) that occur during 3 hours of PCM cooling and to compare them with a CWI treatment of matched temperature. It was hypothesised that both PCM and CWI would decrease intramuscular and core temperature during treatment but with a prolonged effect from PCM due to its ability to deliver a longer cooling duration.

6.2 Methods

6.2.1 Participants

Using the findings of previous studies that examined the change in intramuscular temperature during CWI administered at rest (Gregson et al., 2011) a power calculation was conducted to detect a practically significant decline in intramuscular temperature from CWI or PCM in order to determine an adequate sample size for this study. Since the CWI temperature to be used in the present study was 15°C, it was estimated that the effects would lie somewhere between those seen at 8°C or 22°C (Gregson et al., 2011). Based on the inter-subject variability in the decline in intramuscular temperature at 1 cm of the vastus lateralis with CWI at 8°C and 22°C it was estimated that 10 subjects per group would provide 80% power to detect a 1°C change in temperature at an alpha level of 0.05 (0.88°C using SD of 0.94; 1.01°C using an SD of 1.07). Furthermore, there will be sufficient power to detect a meaningful difference in temperature between CWI and PCM with the proposed sample size because in a subsequent study the difference between the decline in temperature at 1 cm 30 minutes post CWI at 8°C vs. 22°C was $2.26 \pm 0.98^\circ\text{C}$ (Mawhinney et al., 2017).

Eleven physically active males (mean \pm SD; age, 27 ± 6 years; height, 183.6 ± 8.5 cm; body mass, 81.5 ± 12.4 kg) volunteered to participate in this study. Participant recruitment was based on the details described in section 3.2. All participants were free from lower leg injury for at least 1 month prior to study completion and had no known vascular disease in the lower limbs, compromised circulation, allergy or hypersensitivity to cold. Participants were instructed to refrain from strenuous exercise for 72 hours prior to, and for the duration of the study period. The institutional research ethics committee, in line with the Declaration of Helsinki, approved all procedures.

6.2.2 Experimental Design

In this repeated measures, crossover design study participants visited the laboratory on 3 consecutive days. Firstly for a familiarisation session before data collection commenced, followed by two separate treatment sessions, all separated by 24 hours. Participants were randomised to receive one treatment on day 1 and the other treatment on day 2. During PCM treatment, frozen 15°C PCM were applied to the skin over the quadriceps of both legs for 3 hours (Figure 14a). During CWI treatment, participants sat immersed to the umbilicus in an inflatable, temperature-controlled ($15 \pm 1^\circ\text{C}$) cold-water bath (iCool Sport, Australia) for 15 minutes (Figure 14b). Upon completion of PCM treatment, the dry shorts remained on the participant, while rolled up so that the skin remained exposed, for the duration of the recovery period. Upon completion of the CWI, participants were allowed to dry off by patting dry with a towel and change into a new pair of dry shorts which were then rolled up so that the skin remained exposed for the duration of the recovery period. The duration of changing during the CWI session lasted no longer than 5 minutes for each participant.



Figure 14: a) Participant fully instrumented during PCM treatment; and b) during CWI treatment

Vastus lateralis muscle temperature at 1, and 3 cm, skin temperature, core temperature, HR, blood pressure (BP) and thermal comfort were recorded continuously throughout baseline, treatment (15 minutes CWI vs 3 hours PCM) and recovery (2 hours CWI vs 1 hour PCM) during both treatment conditions. Data collection during CWI treatment and recovery consisted of a shorter overall collection period compared to the PCM trial. Since both treatments were matched for temperature, it was impractical for participants to remain instrumented for the additional 1 hour of recovery following CWI treatment, in order to match the duration of PCM treatment and recovery. Thus, recovery of all variables was monitored for 1 hour following completion of PCM treatment (4 hours total time), and for 2 hours following immersion (2 hours 15 minutes total time; see Figure 15). During data collection, participants remained in a semi-reclined seated position with legs outstretched on a bed except during the CWI treatment. All testing was performed in a temperature-controlled laboratory ($24.9 \pm 3.4^{\circ}\text{C}$).

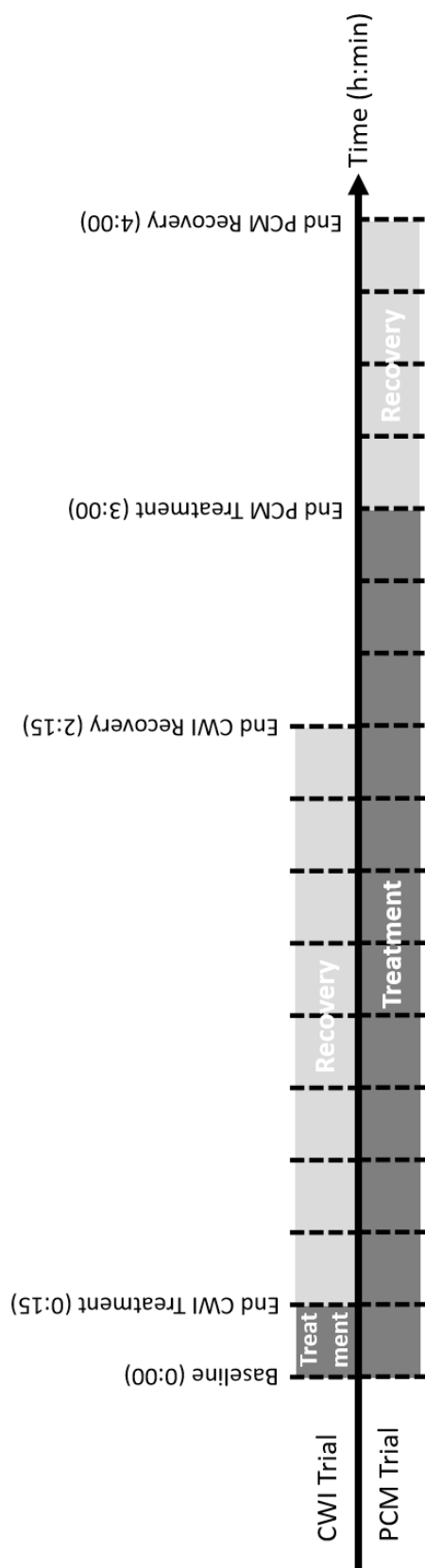


Figure 15: Experimental protocol of treatment and recovery during both conditions over time. Variables recorded every 15 min: Intramuscular, Skin & Core Temp, Blood Pressure, Heart Rate

6.2.3 Phase Change Material Application

For complete PCM application procedures, please refer to section 3.5.

6.2.4 Intramuscular Temperature

To account for subcutaneous fat, skinfold at the exact site of thermocouple insertion on the quadriceps was measured using Skinfold Calipers (Harpenden, Baly International, West Sussex, UK) by the same individual 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 vastus lateralis was then marked approximately 6 cm lateral to the mid-point between the superior pole of the patella and the anterior-superior iliac crest using a sterile pen. Additional markings were placed 1 cm inferior and superior to this point, one for each insertion depth. The area was cleaned with a povidone-iodine surgical scrub solution. Insertion depth was based upon halving the skinfold measure and adding this to the required depth (1 or 3 cm).

A 45 and 32 mm sterile intravenous 20 gauge needle catheter was used for the 3 and 1 cm insertion, respectively. Insertion depth was verified by subtracting the total insertion depth (1 cm or 3cm plus half the skinfold) from the corresponding length of the needle. The difference (length of needle minus calculated insertion depth) was verified with a sterile ruler. Once at the correct insertion depth, the needle was removed, and the flexible catheter remained inserted. A sterile flexible intramuscular Thermocouple Probe (Type T, IT-21; Physitemp Instruments, Clifton, NJ) was threaded through the barrel of the catheter. The catheter was removed from the muscle while the thermocouple remained inserted (Figure 16). The thermocouple insertion site was secured in place with sterile tegaderm by bending the thermocouple flush with the skin. The procedure was then repeated for the 1 cm deep thermocouple. Once fully instrumented, the thermocouples were connected to a digital monitor (Bailey Instruments BAT-12, Physitemp Instruments, Inc.) for continuous recording. Thermocouples remained inserted throughout the duration of treatment and recovery. At the conclusion of data collection, thermocouples were removed, and 'actual' insertion depth was verified by measuring the inserted portion of the thermocouple against a sterile ruler. The left leg of each participant was instrumented with thermocouples for CWI, while the right leg was instrumented for PCM treatment.



Figure 16: *One thermocouple inserted into vastus lateralis showing flexible catheter (pink) threaded over the thermocouple towards the thermocouple 'head' away from the insertion site. The thermocouple head was later plugged into an extension cable and connected to the digital monitor once fully instrumented. A second thermocouple was subsequently inserted as described above (section 6.2.3), and both insertion sites were secured with sterile tegaderm prior to initiation of both treatments.*

6.2.5 Core Temperature

Participants were provided with an activated ingestible core temperature sensor (VitalSense, Respiroic Inc, Murrysville, PA, USA) during familiarisation. Participants were instructed to ingest the capsule with water ~8 hours prior to initial testing. Participants were given a second core temperature sensor following completion of testing on day 1 to ingest at the same time of day as done prior to the first visit. The ingestible core temperature sensor wirelessly transmits core body temperature as it travels through the digestive tract. The sensor's signal passes through the body to the data recorder found on the SEM also used for recording skin temperature data (Hidalgo Ltd, Cambridge, UK). The core temperature sensor passes through the body

at the subject's normal rate of motility. Since core temperature might be influenced by the temperature of ingested material, participants were prohibited from consuming any food or beverage throughout the duration of data collection. Numerous studies have measured core temperature via a telemetric pill and have previously reported this method to be a valid estimate of core body temperature and highly reliable in a sports medicine setting (Byrne & Lim, 2007; Gant, Atkinson, & Williams, 2006; Ganio et al., 2009)

6.2.6 *Skin Temperature*

On arrival to the lab for the treatments, the skin temperature sensor was applied to the quadriceps of the leg that was not being instrumented with intramuscular thermocouples. For detailed procedures to assess skin temperature, please refer to section 3.6.

6.2.7 *Cardiovascular Measures*

Participants were fitted with a wireless ambulatory chest strap HR monitor (EQ02 LifeMonitor, Hidalgo Ltd, Cambridge, UK) that continuously recorded HR. The chest strap housed the SEM data recorder which collected core and skin temperature data. HR data were analysed using Vivosense (Vivonoetics, San Diego, USA). An automatic artefact-marking algorithm was applied to the raw electrocardiogram (sensitivity level: medium noise filtering; minimal and maximal allowable HR limits: 30 and 220 beats per minutes, respectively). R-wave markings were generated for HRV calculations. The square root of the mean squared differences of successive intervals (RMSSD) was reported because RMSSD provides the most reliable and practically applicable measure for day-to-day monitoring (Al Haddad, Laursen, Chollet, Ahmaidi, & Buchheit, 2011). Five minutes rolling averages were calculated for RMSSD, with the baseline measure taken prior to insertion of the intramuscular thermocouples. An automated blood pressure cuff was placed on the participant's right arm (M10-IT; Omron Healthcare).

6.2.8 *Thermal Comfort*

Ratings of thermal comfort were recorded every 15 min. During CWI, thermal comfort was asked at the first and last minute of immersion. Participants were asked to rate their thermal comfort on a nine-point standard scale (Davey, Reilly, Newton, & Tipton, 2007).

6.2.9 Statistical Analysis

All data are presented as mean \pm SD. Within each treatment, the changes in dependent variables over time were assessed by a one factor ANOVA with differences vs baseline assessed using Bonferroni corrections. Over time, the comparison of treatments was assessed using a 2×10 treatment by time repeated measures ANOVA. The two levels for the treatment factor were CWI or PCM. The ten levels for the time factor were baseline [0 minutes], and every 15 minutes up to 2 hours 15 minutes. For these analyses, the entire duration of CWI treatment (15 minutes) and recovery (2 hours) was compared to the first 2 hours 15 minutes of PCM treatment. Recovery effect (return to baseline) from both treatments over time was assessed using a 2×5 treatment by time repeated measures ANOVA. The five levels for the time factor were baseline (0 hours), 15, 30, 45 minutes, 1 hour, and 1 hour 15 minutes for CWI and baseline (0 hours), 3 hours, 3 hours 15 minutes, 3 hours 30 minutes, 3 hours 45 minutes, and 4 hours for PCM. The first 1-hour duration of recovery following each treatment was compared for these analyses. Where there was a significant treatment effect or treatment by time interaction, differences between treatments at any particular time interval were assessed using Bonferroni corrections for planned pairwise comparisons. Additionally, Pearson product-moment correlation coefficients were used to assess the relationship between thigh skinfold thickness and intramuscular temperature.

To estimate the magnitude of the treatment effects, Cohen's d effect sizes (ES) were calculated with the magnitude of effects considered either small (0.20-0.49), medium (0.50-0.79), and large (>0.80). Prior to employing ANOVAs, normality of distribution of all data sets were verified using the Shapiro-Wilk test. Mauchly's test of sphericity was used to assess assumptions of sphericity and, where necessary, Greenhouse-Geisser corrections were applied to tests of within-participant effects. Statistical analyses were performed using SPSS v.21 (IBM, Armonk, NY, USA) and a P-value of less than 0.05 was considered statistically significant.

6.3 Results

6.3.1 Thermocouple Depth and Skinfold

Average skinfolds were not different between legs ($P = 0.377$; right leg: 10.1 ± 5.2 mm, left leg: 9.7 ± 5.5 mm). Thermocouple depths, corrected for skinfolds were 3.0 ± 0.4 cm and 1.0 ± 0.3 cm for PCM and 3.1 ± 0.3 cm and 1.1 ± 0.3 cm for CWI, and were not different between treatments at 3 cm ($P = 0.416$) or 1 cm ($P = 0.688$).

Decreases in intramuscular temperature were correlated with skinfold thickness, with stronger effects at 1 cm (CWI $P < 0.001$, $r = 0.912$; PCM $P < 0.001$, $r = 0.853$) vs. at 3 cm (CWI $P < 0.01$, $r = 0.727$; PCM $P = 0.05$, $r = 0.594$).

6.3.2 Intramuscular Temperature

Intramuscular temperature declined progressively at both depths during both treatments (Table 6; time effect: all $P < 0.0001$). At the conclusion of the recovery period intramuscular temperature remained below baseline at both depths in both treatments (time effect: all $P < 0.0001$).

Table 6: Comparison of vastus lateralis intramuscular temperatures (1cm and 3cm) during baseline, treatment, and recovery of the two cryotherapy treatments (CWI vs PCM).

	CWI 1cm (°C)	CWI 3cm (°C)	PCM 1cm (°C)	PCM 3cm (°C)
Baseline	34.0 ± 1.1	35.6 ± 0.6	33.9 ± 1.5	35.8 ± 0.5
End of Treatment	26.2 ± 2.9	28.4 ± 2.7	26.0 ± 2.2	28.2 ± 2.8
End of Recovery	30.5 ± 1.0	31.0 ± 1.0	29.0 ± 1.6	30.1 ± 2.1
Average	29.4 ± 1.1	30.2 ± 1.2	27.4 ± 2.1\$	29.8 ± 2.4

Note: Values are mean ± SD. Average intramuscular temperature at 1cm was lower from PCM treatment than CWI ($F = 12.4$, $P = 0.006$, $ES = 2.23$; PCM CI: 26.6-29.8°C, CWI CI: 29.1-30.6°C), but there was no difference at 3cm ($P = 0.545$).

Overall intramuscular temperature at both depths was lower from PCM treatment (Figure 17; $F = 113.5$, $P < 0.0001$, $ES = 6.74$; PCM CI: 28.3-30.9°C, CWI CI: 29.6-31.0°C). At the end of CWI treatment intramuscular temperature decreased more rapidly and was 14.0% lower at 1cm and 16.1% lower at 3cm vs 15 minutes into PCM treatment (mean difference at 1 cm: $4.3 \pm 1.7^\circ\text{C}$, $P < 0.0001$, $ES = 2.48$, CI: 3.1-5.4°C; at 3 cm: $5.5 \pm 2.1^\circ\text{C}$, $P < 0.0001$, $ES = 2.61$, CI: 4.1-6.9°C). At 3 cm, intramuscular temperature remained 10.6% lower 15 minutes into recovery following CWI vs 30 minutes into PCM treatment (difference: $3.4 \pm 1.6^\circ\text{C}$, $P = 0.01$, $ES = 2.10$, CI: 2.3-4.5°C), but no longer at 1 cm (2.1%; difference: $0.6 \pm 1.8^\circ\text{C}$, $P = 0.99$). Upon conclusion of CWI recovery (2 hours 15 minutes total time), intramuscular temperature at 3 cm was 7.5% higher compared with 2 hours 15 minutes into PCM

treatment (difference: $2.4 \pm 2.3^{\circ}\text{C}$, $P = 0.045$, $ES = 0.82$, $CI: 3.4-0.3^{\circ}\text{C}$), while intramuscular temperature at 1 cm was on average 12.5% higher during CWI recovery vs PCM treatment from 1 hour to 2 hours 15 minutes ($F = 91.4$, $P < 0.0001$, $ES = 6.03$, $CI: 4.6-2.1^{\circ}\text{C}$).

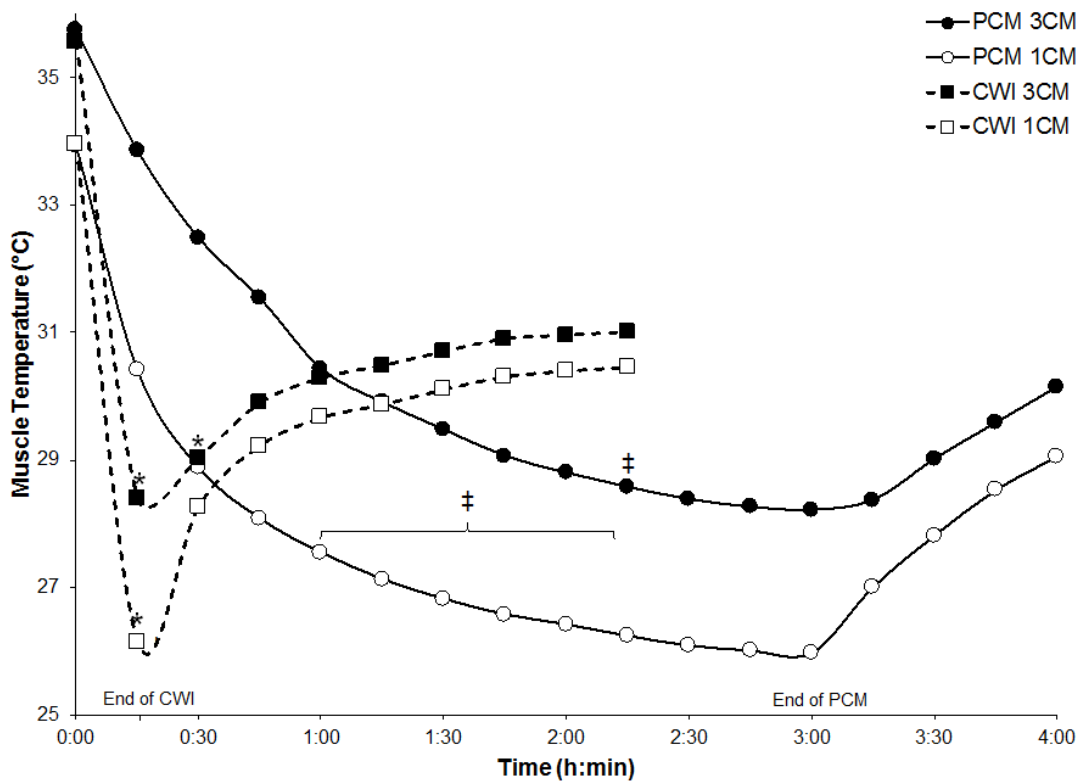


Figure 17: *Vastus lateralis* intramuscular temperature at 1cm and 3cm during both PCM and CWI treatments and for the full duration of recovery from both treatments. Values are mean \pm SD. Intramuscular temperature declined progressively during the PCM treatment ($P < 0.0001$) and remained below baseline after 1:00 of recovery at both depths (1cm: $P < 0.0001$; 3cm: $P < 0.0001$). Intramuscular temperature decreased from baseline immediately following CWI ($P < 0.0001$) and remained below baseline after 2 hours of recovery at both depths (1cm: $P < 0.0001$; 3cm: $P < 0.0001$). Intramuscular temperature was lower with CWI vs PCM at 3 cm from 0:00 to 0:30 (0:15 * $P < 0.0001$; 0:30 * $P < 0.0001$) but only from 0:00 to 0:15 at 1 cm (* $P < 0.0001$). At 3 cm intramuscular temperature was lower with PCM vs CWI at 2:15 ($\pm P = 0.045$), while at 1 cm intramuscular temperature was lower from 1:00 to 2:15 (treatment by time $\pm P < 0.0001$; time effect 1:00 $P = 0.018$; 1:15 $P = 0.001$; 1:30 $P = 0.001$; 1:45 $P = 0.001$; 2:00 $P = 0.001$; 2:15 $P = 0.001$).

When comparing intramuscular temperature for the first 1 hour of recovery from both treatments, the intramuscular temperature at 1 cm was 4.1% higher from CWI averaging $28.6 \pm 1.4^\circ\text{C}$ than from PCM averaging $27.7 \pm 1.7^\circ\text{C}$ (Figure 18; $F = 9.1$, $P = 0.013$, $ES = 1.91$; PCM CI: $25.6\text{-}28.8^\circ\text{C}$, CWI CI: $27.7\text{-}29.6^\circ\text{C}$). However, there was no difference at 3 cm (2.2% ; $P = 0.350$).

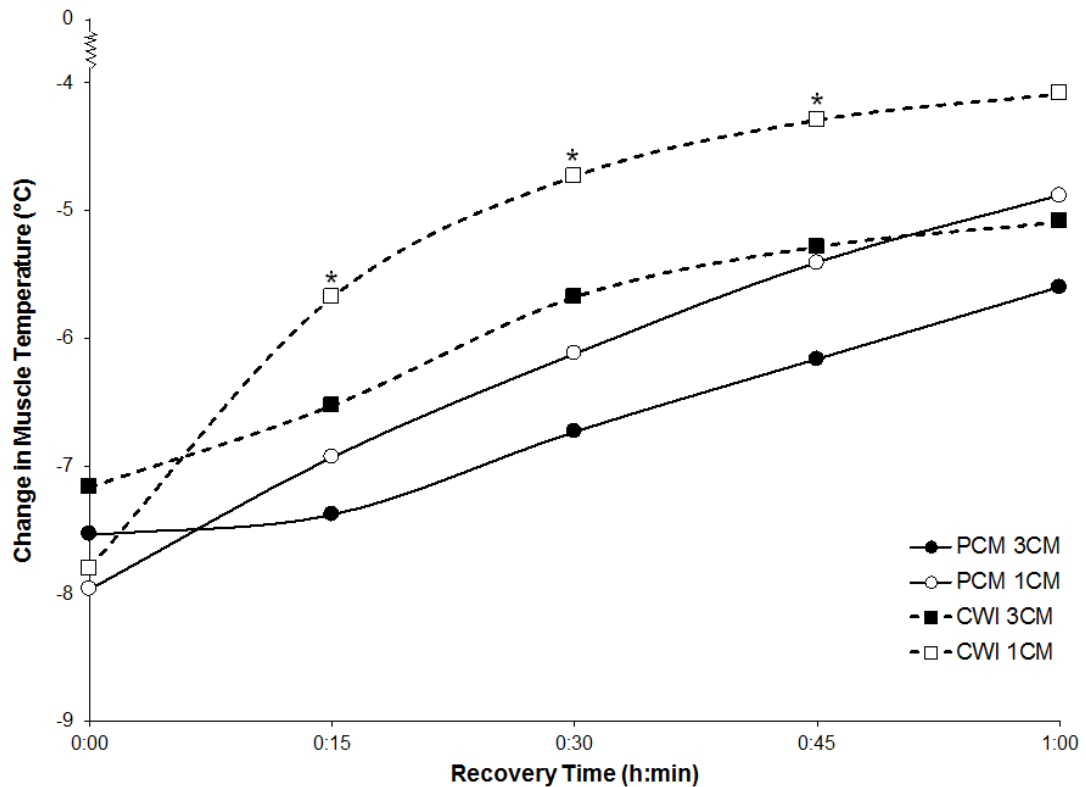


Figure 18: Change in vastus lateralis intramuscular temperature from baseline during the first hour immediately following the conclusion of both PCM and CWI treatments (absolute time displayed for CWI is 0:15 through 1:15, and for PCM is 3:00 through 4:00). Values are mean \pm SD. Overall intramuscular temperature during recovery was lower from PCM than CWI at 1 cm ($P = 0.013$). This difference was specifically evident at 0:15 ($*P = 0.03$, CI: -2.1 to -0.4°C), 0:30 ($*P = 0.005$, CI: -2.1 to -0.7), and 0:45 minutes ($*P = 0.01$, CI: -1.8 to -0.5).

6.3.3 Core Temperature

Core temperature declined during both PCM and CWI treatments (Figure 19; $F = 9.7$, $P < 0.0001$, $ES = 1.98$). Overall the change in core temperature was independent of treatment with no group ($P = 0.741$) or interaction effect observed ($P = 0.100$). The nadir of core temperature from PCM treatment occurred 45 minutes into the recovery period (absolute time: 3 hours 45 min; $0.28 \pm 0.27^\circ\text{C}$ below baseline) while the nadir of core temperature from CWI treatment occurred 1 hour 30 minutes into the recovery period (absolute time: 1 hour 45 min; $0.25 \pm 0.32^\circ\text{C}$ below baseline).

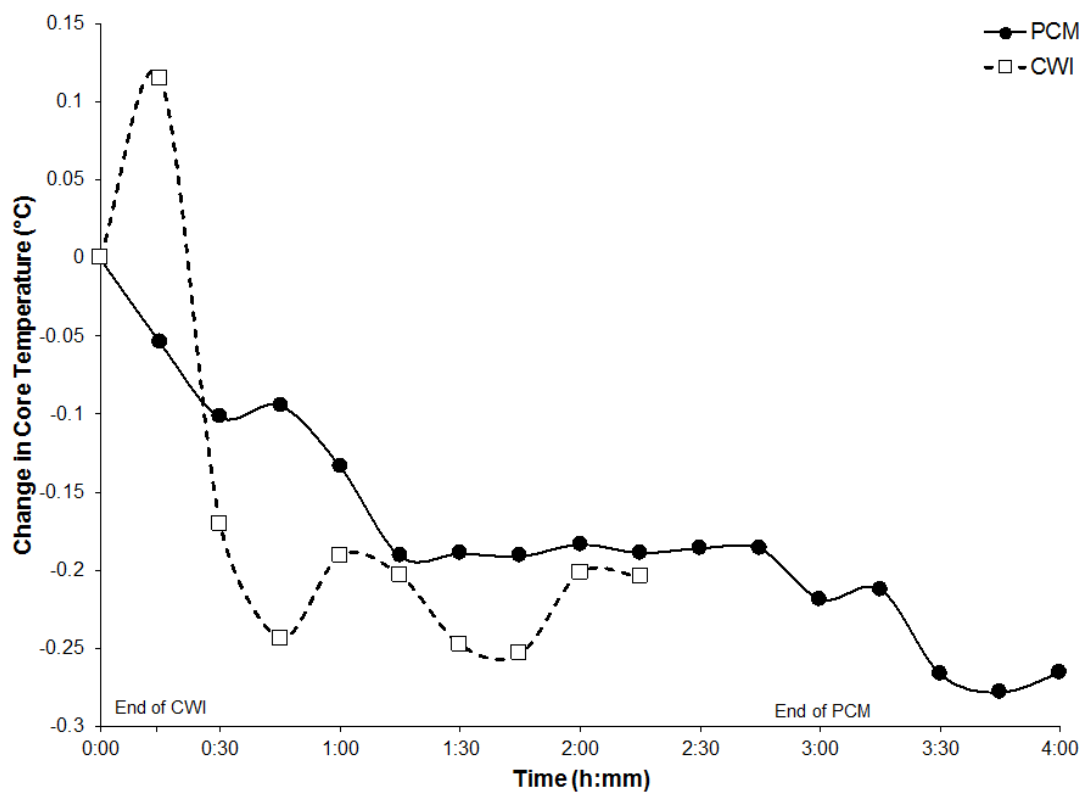


Figure 19: Mean change from baseline in core temperature during treatment and recovery from both PCM and CWI. Values are mean \pm SD. Core temperature declined during the PCM and CWI treatments ($P < 0.0001$).

6.3.4 Skin Temperature

The effect of both treatments on skin temperature at baseline, treatment and recovery are detailed in Table 7. Skin temperature declined during both PCM and CWI treatments ($F = 233.9$, $P < 0.0001$, $ES = 9.67$). CWI decreased skin temperature more rapidly than PCM ($F = 174.4$, $P < 0.0001$, $ES = 8.37$), but the skin temperature was overall lower from PCM treatment than CWI ($F = 124.2$, $P < 0.0001$, $ES = 7.02$; PCM CI: 24.3-25.5°C, CWI CI: 27.8-29.2°C). Skin temperature immediately after CWI was $2.4 \pm 1.7^\circ\text{C}$ lower than 15 minutes into the PCM treatment ($P = 0.01$, $ES = 1.41$, CI: 1.3-3.6°C), however, at all subsequent time points, skin temperature was lower during the PCM treatment (0:30 $P = 0.01$, $ES = 1.50$; 0:45 $P = 0.001$, $ES = 2.65$; 1:00 $P = 0.001$, $ES = 3.74$; 1:15 $P = 0.001$, $ES = 4.18$; 1:30 $P = 0.001$, $ES = 4.54$; 1:45 $P = 0.001$, $ES = 4.78$; 2:00 $P = 0.001$, $ES = 4.73$; 2:15 $P = 0.001$, $ES = 4.77$).

Table 7: A comparison of skin temperatures during baseline, treatment, and recovery of the two cryotherapy treatments (CWI vs PCM).

	CWI (°C)	PCM (°C)
Baseline	31.3 ± 1.1	31.1 ± 0.6
End of Treatment	23.6 ± 0.8	23.8 ± 1.0
End of Recovery	29.5 ± 1.2	27.7 ± 1.1
Treatment Average	-----	24.1 ± 0.3

Note: Values are mean ± SD. CWI does not have a treatment average since data were collected every 15 minutes.

6.3.5 Perceived Thermal Comfort

Overall, thermal comfort was lower for CWI than for PCM ($F = 5.6$, $P = 0.002$, $ES = 1.50$) with greater thermal discomfort reported immediately upon the conclusion of CWI treatment (2.7 ± 0.8 vs. 4.5 ± 0.8 15 minutes into the PCM treatment; $P < 0.0001$, $ES = 1.63$, CI: 1.1-2.6°C). This time point is also where thermal comfort reached its nadir for both treatments. Comparably, thermal comfort was 4.9 ± 1.0 upon conclusion of PCM treatment. Although not different from baseline at any time point during the recovery period, thermal comfort returned to baseline values 30 minutes following PCM treatment (baseline: 5.4 ± 0.8 vs 0:30 recovery: 5.5 ± 1.0). Comparably, thermal

comfort remained below baseline through the first 15 minutes of recovery after CWI ($P = 0.012$, $ES = 3.23$, $CI: 0.2-1.3^{\circ}C$), and did not return to baseline values upon conclusion of data collection (baseline: 5.0 ± 0.8 vs 2:00 recovery: 4.9 ± 1.1).

6.3.6 Cardiovascular Measures

There were technical issues with HR signals for 2 participants during the entire PCM treatment and for one participant after 2 hours of the PCM treatment. Thus, only 9 participants were included in the analysis of HR data, and the analysis only included data for up to 2 hours. HR declined during both treatments (Figure 20; $F = 10.4$, $P < 0.0001$, $ES = 2.04$) with no difference between treatments ($P = 0.151$; Table 8). Overall there was an increase in RMSSD during both treatments ($F = 5.8$, $P < 0.0001$, $ES = 0.65$) with no interaction effect ($P = 0.155$; Table 8). For the PCM treatment there was a trend for an increase in RMSSD ($F = 3.0$, $P = 0.069$, $ES = 1.22$), while for the CWI treatment there was a clear increase in RMSSD ($F = 4.2$, $P < 0.0001$, $ES = 1.45$). Blood pressure was unaffected by either treatment (systolic $P = 0.139$, diastolic $P = 0.904$) and there were no differences between treatments (systolic $P = 0.166$, diastolic $P = 0.101$).

Table 8: Fifteen minute rolling average HR and RMSSD data during 2 hours of PCM application and 15 minutes of CWI followed by 1 hour 45 minutes of recovery.

	<i>Heart Rate (bpm)</i>		<i>RMSSD (ms)</i>	
	<i>PCM</i>	<i>CWI</i>	<i>PCM</i>	<i>CWI</i>
Baseline	68 ± 7	68 ± 8	60 ± 22	62 ± 30
0:15	62 ± 9	61 ± 11	63 ± 24	67 ± 28
0:30	63 ± 7	61 ± 8	61 ± 24	79 ± 25
0:45	64 ± 6	57 ± 8	65 ± 22	74 ± 26
1:00	61 ± 7	59 ± 8	65 ± 26	70 ± 31
1:15	62 ± 9	57 ± 8	70 ± 26	71 ± 27
1:30	60 ± 5	59 ± 11	75 ± 25	75 ± 31
1:45	59 ± 7	56 ± 9	70 ± 25	79 ± 32
2:00	61 ± 9	57 ± 8	73 ± 23	84 ± 33

Note: Values are mean ± SD. HR was reduced over time during both treatments ($P < 0.0001$). There was an increase in RMSSD from CWI ($P < 0.0001$) and a trend for an increase from PCM treatment ($P = 0.069$).

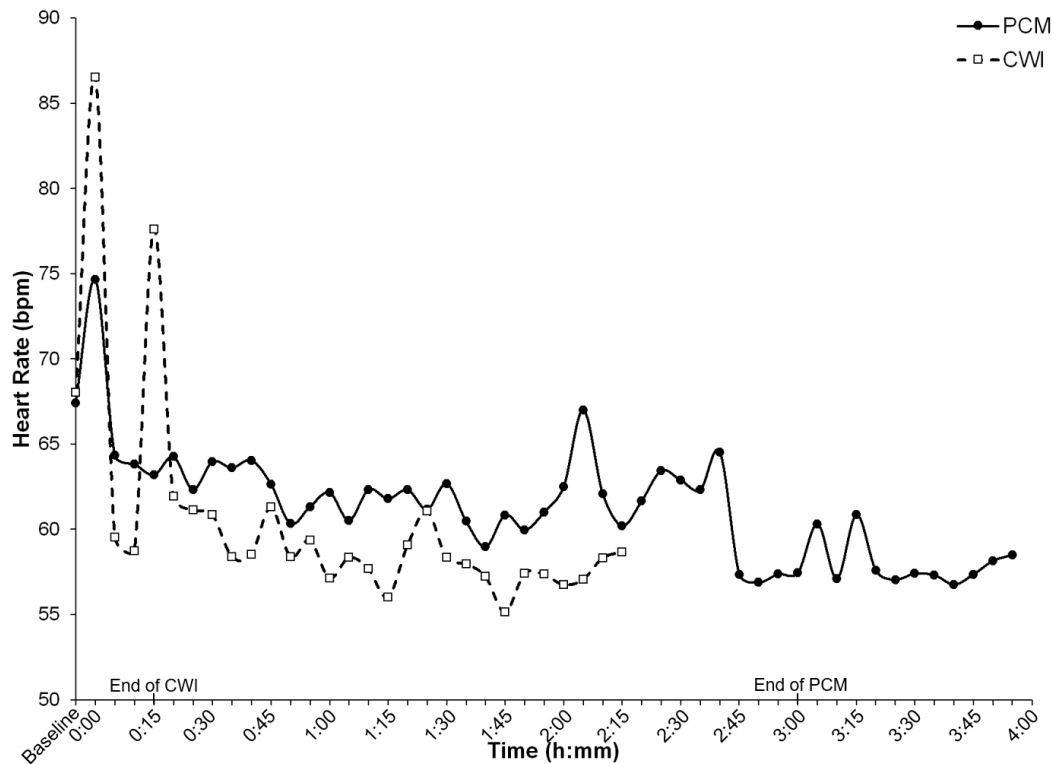


Figure 20: Absolute heart rate during treatment and recovery from both PCM and CWI (in 5 minute intervals). Values are mean \pm SD. Although HR visibly increased during CWI treatment, over time HR declined during both the PCM and CWI treatments and recovery ($P < 0.0001$) with no difference between treatments ($P = 0.151$).

6.4 Discussion

The main finding in this study was that 15 minutes of CWI was comparable to PCM packs applied directly to the skin overlying the quadriceps for 3 hours in terms of the magnitude of reduction in vastus lateralis intramuscular temperature. Ultimately prolonged PCM cooling provided a sustained decrease in intramuscular temperature that was maintained for the 3 hours of application (Figure 17), and a more gradual recovery (Figure 18). However, the initial reduction in intramuscular temperature was more rapid during the CWI treatment. In addition to the local effects on muscle temperature, both treatments delivered local and systemic effects by decreasing core temperature, decreasing HR and increasing HRV. Importantly, the systemic effects were observed despite there being no exercise intervention to induce cardiovascular stress prior to the treatments. The combined local and systemic effects explain in part the accelerated recovery from PCM following exercise of primarily mechanical nature

in chapter 4 as well as in supplementary work (Brownstein et al., 2019; Clifford et al., 2018). Further, the clear systemic effects demonstrated in this study support the response observed in the limb contralateral to the direct cooling limb in chapter 4. This study provides the first evidence that the application of PCM elicits systemic effects, as well as local effects at the musculature comparable to those occurring from CWI treatment.

Current best evidence suggests that to optimise the clinical effectiveness of cryotherapy, a critical level of muscle cooling must be achieved (Bleakley & Hopkins, 2010). Reducing muscle temperature will reduce cellular metabolism which would decrease the proliferation of secondary injury, thereby limiting the overall extent of tissue damage (Knight, 1995; Merrick, 2002). Previously, muscle metabolism was favourably minimised at a temperature range of 10-15°C in animal models (Sapega et al., 1988). Studies in humans have demonstrated reductions in muscle temperature immediately following CWI performed after exercise that range between ~31°C at 1 cm (Mawhinney et al., 2013; Mawhinney et al., 2017), ~28°C at an unknown intramuscular insertion depth (Roberts, Muthalib, et al., 2015), and ~24°C at 1 cm (Roberts et al., 2014). Comparably the lowest absolute intramuscular temperature achieved in this study was 26.0°C at 1 cm from both PCM cooling and CWI. However, in the present study, average vastus lateralis temperature at 1 cm for the total PCM trial period (4 hours) was 7% lower than the average temperature at 1 cm for the total CWI trial (2 hours 15 minutes). Thus, not only can PCM provide prolonged cooling, but it can also provide a greater magnitude of cooling to the peripheral musculature. This may have implications for the preferential use of PCM over CWI in exercise recovery. Especially since the damage that occurs following strenuous exercise is bimodal, involving both the initial mechanical and/or metabolic muscle injury and a secondary phase that involves a disruption in intracellular homeostasis followed by an inflammatory response which initiates 2-6 hours post damaging exercise (Armstrong et al., 1991). Thus a prolonged cooling intervention during this timeframe has potential to blunt the harmful processes that occur during the phase of secondary damage, limiting further haemorrhage and cell death (Schaser et al., 2007). In support of this rationale, it has previously been demonstrated in animal models that local cooling for prolonged durations limited secondary damage (3-6 hours; Merrick et al., 1999; Puntel et al., 2012; Sapega et al., 1988; Schaser et al., 2007).

Few studies have examined the impact of CWI on resting core temperature where there was no exercise-induced temperature elevation prior to CWI treatment (Costello

et al., 2012a; Gregson et al., 2011). Costello et al. (2012) reported a $0.4 \pm 0.2^{\circ}\text{C}$ reduction following 4 minutes of CWI at 8°C while Gregson et al. (2011) reported a $0.2 \pm 0.1^{\circ}\text{C}$ drop in core temperature following two 5 minute treatments in 8°C water. Comparable reductions in core temperature were evident from both treatments in the present study. This finding was surprising considering that PCM was applied locally, while CWI involved submerging a large part of the body. However, the longer treatment duration during PCM cooling delivered a continued decrease in muscle temperature which provided a temperature gradient for heat removal from the central core resulting in a progressive decline in core temperature. It is well understood that the pattern and magnitude of change between peripheral tissue temperature and core temperature differs due to the increased reliance of core temperature change on convective cooling as opposed to conductive cooling (Taylor, Caldwell, van den Heuvel, & Patterson, 2008). CWI is associated with vasoconstriction which suppresses peripheral blood flow and the consequential reduction in convective heat delivery from the core to the periphery which causes a substantial reduction in core temperature. On the contrary, local cryotherapy that induces cutaneous vasoconstriction at the periphery typically limits the systemic exposure of blood to cold tissues, and thus core temperature is often unaffected. However, as was evident by the reduction in core temperature in the PCM condition in present study, it was likely that blood flow to skin was maintained during PCM treatment. This effect likely sufficiently exposed the circulating blood to the cold tissues allowing for a comparable reduction in convective heat delivery to occur from the core to the periphery during the application period. It is likely that a similar effect would not be observed in the PCM treatment condition following exercise as the local cooling stimulus might be too little to influence convective cooling. Previous studies have shown that the rate of core temperature reduction during post-exercise CWI is dependent on temperature, duration, the time from the end of exercise to commencement of CWI treatment, and perhaps most importantly that intermittent protocols result in a significantly greater decrement in core temperature compared to continuous protocols (Stephens, Sharpe, Gore, Miller, Slater, et al., 2018). Therefore, the results of the present study may not translate directly to an exercise paradigm.

Monitoring indices of HRV, as indicators of autonomic nervous system activity, has been of increasing interest among athletes (Stephens, Halson, Miller, Slater, & Askew, 2018). This is because during exercise sympathetic activity is increased, while a concomitant progressive parasympathetic reactivation occurs in the post-exercise period with a sympathetic withdrawal (Al Haddad et al., 2010). In over-

trained or under-recovered athletes an imbalance can occur in this cycle. Therefore the restoration of cardiovascular homeostasis is an important component of overall recovery and interventions that increase HRV are thought to be advantageous to exercise recovery (Dong, 2016). Post-exercise CWI has been shown to accelerate recovery of HRV (Al Haddad et al., 2010; Almeida et al., 2016). Both authors concluded that water immersion is a simple and efficient means of triggering post-exercise parasympathetic reactivation, lowering sympathetic tone, and restoring cardiac autonomic modulation. In the present study there was a decrease in HR and an increase in HRV from both treatments. The mechanisms with which both CWI and PCM stimulated this response are likely related to the effect of vasoconstriction. Exposure to cryotherapy reduces HR as a result of increased stroke volume, which may be a result of cold-induced vasoconstriction. The finding that both PCM and CWI reduced HR and HRV was again surprising considering that PCM was applied locally. Theoretically CWI should have had a greater effect on cardiac parasympathetic activity compared with the PCM treatment, due to the systemic as opposed to local vasoconstriction that can induce greater increases in central blood volume or faster reductions in core temperature (Buchheit, Peiffer, Abbiss, & Laursen, 2009). As the reductions in core temperature were comparable across treatments it was evident that, under resting conditions, the local cryotherapy stimulus occurring from PCM cooling was enough to cause systemic effects. However, it remains imperative to again mention that this effect would likely have been suppressed following exercise.

An interesting observation in the present study was the very clear divers reflex and Hunting reaction evident during the CWI treatment, but not during the PCM treatment. This phenomenon is brought about when superficial temperature rapidly drops and there is an initial re-distribution of warm blood from the periphery to the core in order to enable the preservation of core temperature. This rise in core temperature was apparent during the CWI treatment, which resulted in an increase in core temperature by 0.115°C (see Figure 19). This rise in core temperature corresponds with the elevated HR evident both immediately upon the commencement and cessation of CWI treatment, but not during PCM treatment (see Figure 20). As evident by the rise in core temperature during the CWI treatment, cold-induced vasoconstriction likely redistributed the peripheral blood into the thoracic vasculature, thereby increasing central blood volume and cardiac output which increased the HR during treatment. Indeed, this increase in HR, and the very likely accompanying increase in mean arterial blood pressure, is consistent with the cardiovascular cold pressor response (Lovallo, 1975; Raven, Wilkerson, Horvath, & Bolduan, 1975; Wray, Donato,

Nishiyama, & Richardson, 2007). Although HR was also slightly increased upon the initiation of PCM treatment, the spike was smaller. These results may have implications in athletes who already have elevated HRs following exercise. Furthermore, it is unknown what effect the periodic dilation of superficial tissues and warming of the core temperature during CWI treatment may have on recovery from exercise. However recent work investigating the effect of CWI on blood flow following exercise suggests that the results evident in the present study would not occur to similar magnitudes under exercising conditions (Mawhinney et al., 2013). The authors suggested that although the increased body temperature in individuals following exercise may reduce the overall degree of vasoconstriction, the vasoconstrictive response would be maintained throughout immersion, preventing the associated onset of cold-induced vasodilation.

Since this study did not utilise an exercise intervention prior to the treatments, both the magnitude and duration of the physiological effects cannot be extrapolated to what might occur in a post-exercise condition. Indeed, the rate of reduction in blood flow from cryotherapy is inversely related to the activity status of the individual; meaning that a reduction in blood flow from cryotherapy is less following exercise than at rest (Gregson et al., 2011; Ihsan et al., 2013; Mawhinney et al., 2013; Yanagisawa, Kudo, Takahashi, & Yoshioka, 2004). Gregson et al. (2011) have previously demonstrated that colder immersion temperatures result in a larger cold-induced vasodilation response and thus higher cutaneous blood flow at rest. The authors explained that under resting conditions, increases in cutaneous blood flow during colder water immersion redistribute blood from the underlying muscle; consequently, colder water may be more effective in reducing muscle blood flow and inflammation at rest. However, this response is not the same following exercise. Exercise increases body temperature and skin blood flow. The elevations in thermal load following exercise ultimately reduce the magnitude of vasoconstriction that occurs during CWI treatment (Mawhinney et al., 2013). Thus, the cryotherapy dose must be greater following exercise than what would be necessary at rest in order to induce significant changes in blood flow following exercise. Therefore, the reductions in muscle and core temperature evident in the present study may not be replicated or might occur at a lesser degree following exercise encompassing a substantial metabolic component. This might explain the lack of effect from PCM cooling on accelerating recovery following a marathon run demonstrated in chapter 5. The extreme metabolic stress experienced by the entire body following the marathon likely elevated muscle and core temperatures in the study participants more so than in the

participants in chapter 4. In this regard local application of PCM to one muscle group in marathon runners might have been too little of a cooling stimulus to elicit any beneficial recovery effects.

This study was not without limitations. Firstly, the shorter overall data collection period during the CWI trial compared with the PCM trial complicated the comparisons between treatments. However, it was not practical to have study participants remain instrumented for an additional 1 hour 45 minutes following CWI to match the PCM duration, especially since it was a crossover design. A post CWI duration of 2 hours was sufficient to demonstrate the magnitude and duration of effects on recovery, especially since it has been demonstrated that intramuscular temperature does not return to baseline for up to 4 hours following CWI administered after exercise (Johnson, Moore, Moore, & Oliver, 1979). The 3 hours PCM duration was chosen to replicate the treatment time in field testing (Clifford et al., 2018), and the 1 hour recovery time was deemed sufficient and practical for study participants who were sitting for more than 4 hours. Previous studies have demonstrated that the cooling effect in calf muscles is maintained for 3-4 hours following CWI in normothermic individuals due to inactivity (Myrer et al., 2001; Oliver & Johnson, 1976). Therefore, it was not feasible to keep study participants instrumented to monitor temperatures that likely would not have returned to baseline.

Secondly, this study utilised a cohort of male participants with thigh skinfolds averaging 9.9 ± 5.2 mm. Reductions in intramuscular temperature were correlated with skinfold thickness during both treatments, due to the insulating effect of adiposity (Otte et al., 2002; Stephens et al., 2017). It has previously been shown that body composition influences the magnitude of change in the skin, muscle, and core temperature during and after CWI (Stephens, Halson, Miller, Slater, & Askew, 2018). It has also been suggested that muscle mass and its regional distribution, body surface area to mass ratio, age and ethnicity influence thermal and physiological responses to water immersion (Stephens et al., 2017). Therefore, the results of this study should be cautiously interpreted when relating them to a more heterogeneous group. This study should be repeated in a female population since women generally have greater subcutaneous body fat compared to men, and because for a given change in body temperature, as occurs during and from exercise, females require a greater cooling stimulus to maintain thermal comfort levels (Vargas et al., 2019). The results from this study might further differ in a female population due to the added variable of sex hormone-related fluctuations in body temperature and some

thermoregulatory processes during the menstrual cycle (Kaciuba-Uscilko & Grucza, 2001). Consequently, the results of this study should be extrapolated with a degree of caution to the effect of CWI or PCM on intramuscular and core temperature in females and following exercise in both genders.

6.5 Conclusion

This was the first examination of the effect of PCM cooling on intramuscular temperature, core temperature and cardiovascular function. The magnitude of temperature reduction from prolonged PCM application was similar to that occurring during CWI treatment, but critically, PCM provided a cooling effect that was sustained for the entire duration of treatment and was better tolerated than CWI. The physiological effects evident in the present chapter might explain the previously reported benefits of PCM cooling in reducing muscle damage and accelerating recovery following eccentric exercise (chapter 4) as well as in professional soccer players (Brownstein et al., 2019; Clifford et al., 2018). However, as the present study implemented both treatments in resting individuals, future research should examine the impact of PCM application on intramuscular and core temperatures following exercise of both mechanical and metabolic nature, as well as in a more heterogeneous group.

6.6 Perspectives

This chapter aimed to determine the effects from 3 hours of PCM cooling and 15 minutes of CWI, controlled for treatment temperature (15°C PCM and $15 \pm 1^{\circ}\text{C}$ CWI), on the intramuscular, core, and skin-temperature and cardiovascular responses. Although the magnitude of temperature reduction from both PCM and CWI was comparable, CWI reduced intramuscular temperature faster while PCM maintained the reduction in intramuscular temperature for longer (throughout the duration of PCM treatment). Importantly, this chapter demonstrated that both superficial (1cm) and deep (3cm) intramuscular temperatures remained reduced for the full duration of PCM treatment. These findings, in addition to the favourable reductions in skin and core temperature, indicate that prolonged exposure to cold via PCM does not result in thermal discomfort or risk of cold-related injury. This study further confirmed that, during application, both cryotherapy modalities exerted a central effect on core temperature and HR. These results suggest that the beneficial effect observed from indirect PCM cooling in chapter 4 was indeed a true systemic effect.

The findings of the present study, that both PCM and CWI reduce intramuscular temperatures to the same degree, support the use of PCM for prolonging the duration of cryotherapy exposure in order to prolong the effects on muscle and core temperature. The results from this investigation suggest that prolonged PCM is an efficacious alternative to CWI, in terms of reducing intramuscular and core temperatures. However, the present study utilised a thermoneutral non-exercising model. Thus, whether similar reductions in muscle and core temperatures would occur in exercising individuals can not be directly inferred from these results. Nevertheless, based on the known thermal and cardiovascular responses to CWI following exercise (Stephens et al., 2017), it is fair to assume that the physiological effects evident in the present study would have occurred to a lesser degree in the previous two chapters (due to the larger thermal gradients apparent following exercise vs in resting individuals). Furthermore, it is likely that the lack of effect in chapter 5 but not in chapter 4 could have been a result of a larger thermal gradient in the participants following the marathon in chapter 5 than following the isolated eccentric exercise in chapter 4. Ultimately, the findings from the present chapter provide evidence to support the overall aim of the thesis, which is to establish the efficacy of PCM as an alternative cryotherapy intervention for recovery from EIMD.

7.0 PROLONGED COOLING WITH PHASE CHANGE MATERIAL ENHANCES RECOVERY AND DOES NOT AFFECT THE SUBSEQUENT REPEATED BOUT EFFECT FOLLOWING EXERCISE

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Kwiecien SY, McHugh MP, Hicks KM, Keane K, Howatson G. (2019). Prolonged cooling with phase change material enhances recovery and does not affect the subsequent repeated bout effect following exercise. *Eur J Appl Physiol.* 120(2), 413-423.

7.1 Introduction

The physiological response occurring during eccentric contractions places high mechanical stress on the involved muscles (see section 2.1.1). Mechanical stress results in EIMD and is accompanied by soreness, decrements in performance such as strength loss, a rise in serum CK concentration and changes in muscle morphology occurring after the acute bout of exercise (Brown et al., 1997; Ebbeling & Clarkson, 1989; Howatson et al., 2007; McHugh et al., 2001, 1999; Nosaka & Clarkson, 1995). However, a prior bout of eccentric exercise provides a rapid adaptation and protection against both primary and secondary muscle damage in subsequent exercise sessions (RBE; Clarkson et al., 1987b; see section 2.1.3). The RBE is a protective effect against further muscle damage (Evans et al., 1986; Hyldahl, Chen, & Nosaka, 2017; McHugh, 2003) as evident by an attenuation in the signs and symptoms of EIMD following a second bout of exercise (Howatson & van Someren, 2008; Nosaka & Clarkson, 1995). However, athletes can still experience muscle damage albeit the stages of muscle damage in trained athletes are shorter, and the overall magnitude of EIMD is less than what it would be in untrained individuals.

Athletes commonly utilise a plethora of recovery modalities to mitigate the acute and chronic signs and symptoms of EIMD, in an effort to recover faster and gain a competitive advantage. Cryotherapy, in the form of CWI, is one of the most popular recovery modalities utilised by athletes for its ability to diminish perceptions of soreness (see section 2.2.5.3.2). However, CWI treatment duration, limited by treatment temperature, is insufficient to markedly influence the physiological mechanisms responsible for accelerating recovery of the signs and symptoms of EIMD. In order to overcome these limitations, the use of a cooling modality capable of prolonging the duration of cryotherapy exposure was introduced in chapter 4. The ability of PCM to safely maintain reduced intramuscular and core temperatures, comparable to CWI, was demonstrated in chapter 6. However, in chapter 4, PCM was applied following only a single session of exercise. Therefore, it remains unknown whether the RBE can still be induced in a subsequent bout of exercise if PCM accelerated recovery after an initial bout of exercise.

Cryotherapy interventions that suppress the acute inflammatory response and accelerate recovery might negatively affect the damage-repair-adaptation processes of skeletal muscle (Woods, Lu, & Lowder, 2000), functional performance and proprioceptive acuity (Costello & Donnelly, 2011), and might hinder normal adaptive training responses to strenuous exercise (Figueiredo et al., 2016; Fröhlich et al.,

2014; Roberts, Raastad, et al., 2015; Yamane, Ohnishi, & Matsumoto, 2015; Yamane et al., 2005). Hence it remains imperative to recognize the paradox between the use of cryotherapy to facilitate recovery and the potential negative effects that may be caused by blunting the stress response (White & Wells, 2013). Especially since some degree of inflammation, which plays a crucial role in the remodeling and adaptation of skeletal muscle is required for the resolution of muscle fibre damage resulting from an exercise insult. Indeed, CWI after routine strength workouts has been shown to attenuate training-induced adaptations during several weeks of training (Yamane et al., 2005). However, the effects of cryotherapy interventions on the RBE and whether they inhibit the RBE are not well understood.

The overall aim of this thesis is to determine whether prolonged PCM cooling can be effectively utilised for recovery following exercise. However, chapter 4 only established the efficacy of prolonged PCM cooling for accelerating recovery following a single exercise exposure. Therefore, it remains imperative to determine its efficacy between repeat bouts of exercise. Hence this chapter aimed to determine whether prolonged PCM cooling after an initial bout of eccentric exercise blunts the adaptive response that protects against damage following a subsequent bout of eccentric exercise. Additionally, in order to examine the effects of prolonged cooling on blood markers of muscle damage and inflammation following exercise of mechanical nature, it was necessary to measure these variables in this chapter as they were not assessed in chapter 4. The hypothesis was twofold: i) that prolonged PCM cooling would accelerate recovery from eccentric exercise compared to a control, and that ii) the accelerated recovery from an initial bout of strenuous quadriceps exercise would reduce the adaptive response to a subsequent bout of strenuous quadriceps exercise.

7.2 Methods

7.2.1 *Participants*

A sample size analyses was performed based on the strength results from chapter 4 and Clifford's (2018) work. It was estimated that a minimum of 13 participants per group were needed to detect a 10% difference in strength with a power of 0.80 and an alpha level of 0.05. Consequently, twenty-six male participants (mean \pm SD; age, 25 \pm 6 years; height, 179.5 \pm 5.6 cm; body mass, 82.9 \pm 11.9 kg) volunteered to participate in this study. All participants were trained athletes participating in sport, e.g. ice hockey, basketball, soccer, Gaelic football, powerlifting. Participants

completed the experimental protocol during their offseason. Participants were free from injury within the past 6 months. Participants were instructed to refrain from taking non-steroidal anti-inflammatory drugs, nutritional supplements, pharmacological interventions, therapeutic interventions, and strenuous exercise unrelated to the present study for the duration of the two study periods. The institutional research ethics committee, in line with the Declaration of Helsinki, approved all procedures.

7.2.2 Experimental Design

Participants reported to the laboratory for two periods of four consecutive days, each period separated by two weeks. On day one participants performed bout 1 of eccentric quadriceps exercise on each leg and were randomised to receive either 'treatment' frozen 15°C PCM or 'control' room temperature PCM applied to the quadriceps for 6 hours, starting immediately following the exercise. Two weeks later participants performed bout 2 and repeated an identical bout of eccentric exercise. All participants received room temperature PCM post-exercise following bout 2. Prior to each eccentric exercise bout, and on each of the subsequent days, assessments of soreness, strength (Newton meters; Nm), and blood markers of muscle damage (CK) and inflammation (hsCRP) were made (Figure 21).

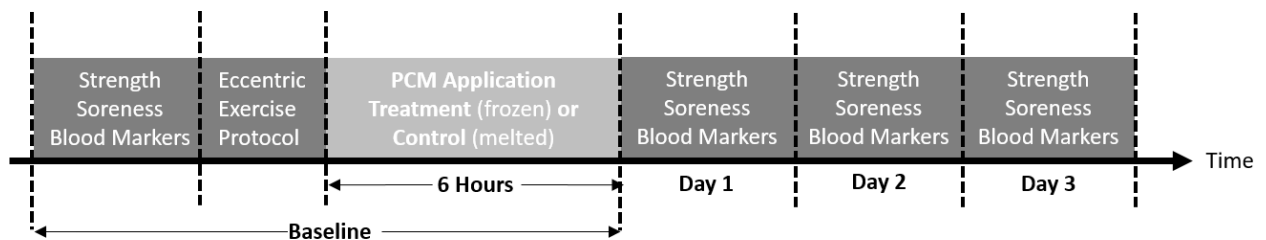


Figure 21: *Experimental protocol for one bout of isolated eccentric quadriceps exercise.* The protocol was repeated for bout two, with the exception that both treatment and control conditions received room temperature PCM following exercise. (Note: figure not to scale).

7.2.3 Phase Change Material Application

Upon initial completion of the eccentric exercise protocol, participants in the treatment group immediately applied frozen 15°C PCM to the quadriceps of both legs while participants in the control group applied melted room temperature PCM to both legs,

for a total of 6 hours. Upon completion of the exercise protocol 2 weeks later, both treatment groups applied room temperature PCM to both legs for a total of 6 hours. For complete PCM application procedures, please refer to section 3.5 and Figure 7.

7.2.4 Strength Assessment

Strength was tested at each of 50°, 80° and 100° knee flexion. For complete strength testing procedures, please refer to section 3.8.

7.2.5 Eccentric Exercise Protocol

The eccentric exercise protocol consisted of 120 eccentric quadriceps contractions (10 sets, 12 repetitions), with the range of motion set at 40° (0° = full extension) to 100° knee flexion. For thorough detail of the eccentric exercise protocol, please refer to section 3.9.

7.2.6 Soreness Assessment

For procedures to assess muscle soreness, please refer to section 3.10.

7.2.7 Skin Temperature Measurement

For procedures to assess skin temperature, please refer to section 3.6.

7.2.8 Compression Pressure Measurement

For procedures to measure compression pressure, please refer to section 3.7.

7.2.9 Blood Sampling and Analysis

For a detailed explanation of blood sampling and analysis, please refer to section 3.11.

7.2.10 Statistical Analysis

All data are presented as mean \pm SD. Since torque differences between angles were not relevant to the effect PCM cooling had on the recovery of strength, strength was reported as the average of each leg at each of the three test angles for assessing strength loss on the days after eccentric exercise. In order to remove the effect of inter-individual variation in knee extension strength, strength on the 3 days following eccentric exercise was expressed as a percentage change relative to baseline strength of each respective bout. The effect of PCM cooling across exercise bouts on average strength loss, soreness, CK, and hsCRP on the days after eccentric exercise

was assessed using 2 x 2 x 4 mixed-model ANOVA. The two levels for the treatment factor were PCM cooling and control. The two levels for the bout factor were initial bout (bout 1) and repeated bout (bout 2). The four levels for the time factor were baseline, day 1, day 2, and day 3 after the eccentric exercise. Strength prior to the initial eccentric exercise bout was compared to strength prior to the repeated bout using 2 x 2 x 3 treatment by bout by angle mixed-model ANOVA. The purpose of these analyses was to see if there was a shift in the angle-torque relationship consistent with the RBE, with a relative increase in torque at long muscle lengths and a decline at short muscle lengths as shown by McHugh & Tetro, (2003). The average skin temperature during each bout was compared between treatments using a mixed-model ANOVA.

Normality of all data sets was examined using the Shapiro-Wilk test and where necessary data were log transformed to establish a normal distribution (CK was the only variable that had to be log transformed). Additionally, CK as a percentage change from baseline was analysed using Friedman Tests for the effect of time and Wilcoxon Signed Ranks Test for pairwise comparisons. Mauchly's test was used to assess assumptions of sphericity and, where necessary, Greenhouse–Geisser corrections were used. Where there was a significant treatment or treatment by time interaction effect, differences between treatments at any particular time interval were assessed with independent t-tests using Bonferroni corrections for planned pairwise comparisons. Where appropriate, Cohen's d ES statistics were calculated to provide magnitude of effects; with the magnitude of effects considered either small (0.20-0.49), medium (0.50-0.79), and large (>0.80). The upper and lower limit of 95% confidence intervals (CI) for least significant difference are reported where relevant. Statistical analyses were performed using SPSS v.21 (IBM, Armonk, NY, USA) and a P-value of less than 0.05 was considered statistically significant.

7.3 Results

7.3.1 Strength

In bout 1 quadriceps muscle strength showed a main effect of time ($F = 9.9$, $P < 0.0001$, $ES = 1.28$; Figure 22), indicating the presence of muscle damage after the initial bout of isolated eccentric exercise. In bout 1 overall recovery of MIVC was independent of treatment ($P = 0.75$); however, the significant interaction effect ($F = 5.5$, $P = 0.002$, $ES = 0.96$) indicated that over time there was a greater recovery of strength in the PCM treatment vs the control group (PCM time effect: $F = 24.6$, $P = <$

0.0001, ES = 2.86, PCM CI: 98.4-103.0%; control time effect: P = 0.143, control CI: 92.1-100.8%). By contrast, quadriceps muscle strength also showed a main effect of time in the repeated bout (F = 4.5, P = 0.011, ES = 0.87), but there was no group (P = 0.058) or interaction effect (P = 0.172). While there was no significant overall bout (P = 0.093) or treatment effect (P = 0.910), there was a significant bout*treatment interaction (F = 5.7, P = 0.025, ES = 0.98; PCM B1 CI: 97.4-104.0%, B2 CI: 96.7-102.4%; control B1 CI: 93.2-99.8%, B2 CI: 100.6-106.4%) and a significant treatment*bout*time interaction (F = 5.4, P = 0.002, ES = 0.95). Expectedly, post hoc analyses indicated that only the control condition exhibited a significant bout effect (F = 6.2, P = 0.028, ES = 1.44, control B1 CI: 92.1-100.8%, B2 CI: 100.3-106.7%; PCM bout effect: P = 0.573).

There was no change in the baseline angle-torque relationship between the initial and repeated bouts (P = 0.90). Further the overall bout by treatment effect at baseline was P = 0.052. However, in the PCM cooling group, baseline strength increased across all three angles by 10% from the initial bout to the repeated bout (F = 17.8, P = 0.001, ES = 2.43, CI: 11.3 to 35.5; increased by 23.4 ± 20 Nm in the second bout but remained unchanged between bouts in the control group (< 1% difference; P = 0.809). For illustrative purposes, torque data for each day of data collection of each bout are presented in Table 9 to demonstrate the return of function following the exercise protocol.

During eccentric exercise in bout 1, average peak torque (Nm) increased by 9% from the first to the 10th set (F = 8.0, P < 0.0001, ES = 1.15), but this was not different between treatments (P = 0.319; 6.5% increase in PCM treatment vs. 11.6% increase in control). In bout 2, eccentric torque was not different from the first to the 10th set (P = 0.515), and there was no difference between treatments (P = 0.142). For bout 1, eccentric torque was 100 ± 1.0% of isometric peak torque for PCM treatment (P = 0.991) and 102 ± 1.0% for control (P = 0.689). For bout 2, eccentric torque was 102 ± 1.2% for PCM treatment (P = 0.564) and 115 ± 1.3% for control (P = 0.032, ES = 0.95). Target intensity over the 10 sets was not different from the initial to the repeated bout for PCM treatment (P = 0.392), but increased by 10% in the control group (F = 9.4, P = 0.011, ES = 1.85; B1 CI: 6.7-12.8%, B2 CI: 5.3-24.5%).

Table 9: Isometric strength (MIVC) of the quadriceps averaged across all 3 test angles of the PCM treatment and control group reported as net torque values (Nm) on each day after eccentric exercise for each bout of exercise.

	PCM (Nm)		Control (Nm)	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline	222 ± 46	246 ± 53	231 ± 46	233 ± 53
Day 1	210 ± 52	238 ± 53	219 ± 52	238 ± 53
Day 2	224 ± 52	244 ± 46	219 ± 52	239 ± 46
Day 3	239 ± 56	248 ± 47	225 ± 56	248 ± 47
<i>Time Effect</i>	P < 0.0001	P = 0.113	P = 0.157	P = 0.061

Note: Values are mean ± SD.

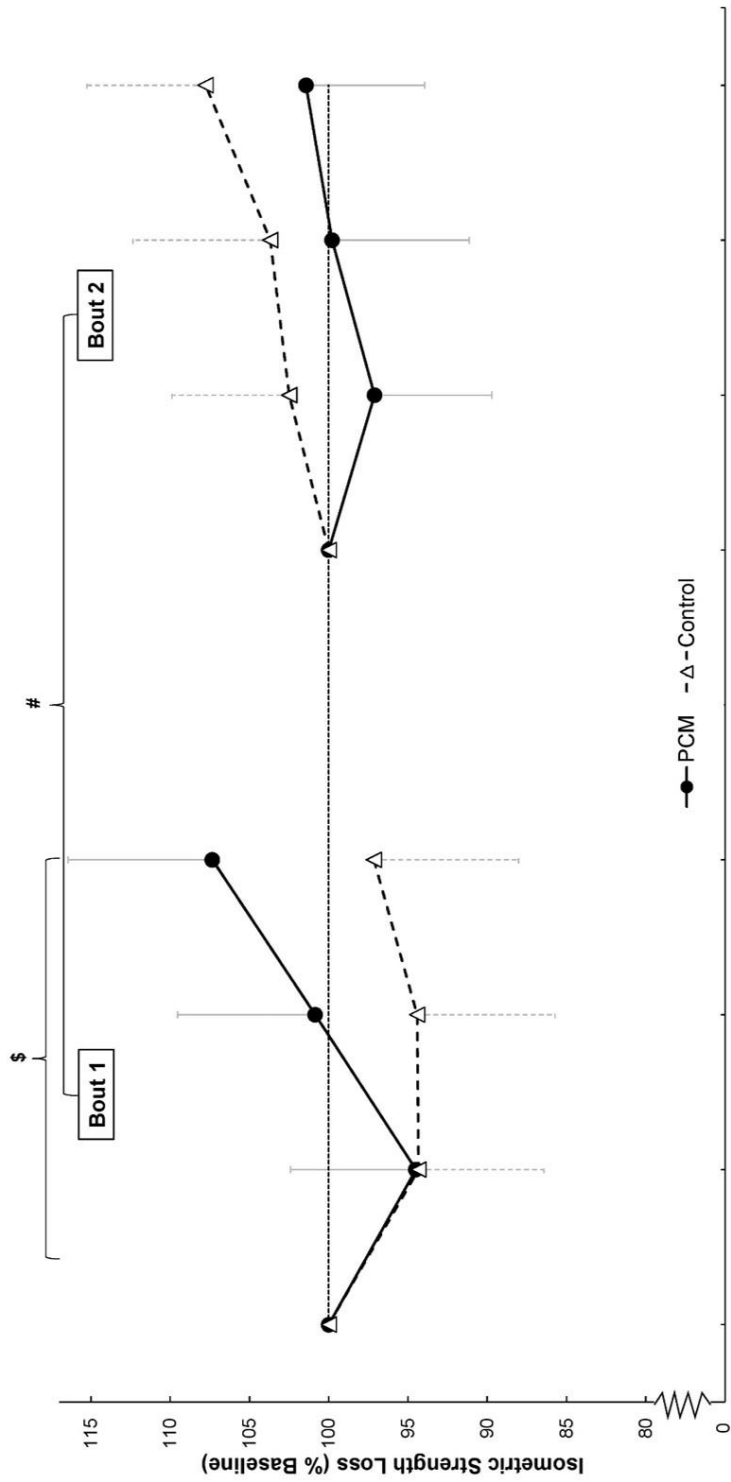


Figure 22: Isometric strength loss of the quadriceps (presented as percent of baseline strength loss) for the PCM treatment and control groups before and on the three days following each bout of eccentric exercise. Values are mean \pm SD. Difference from baseline as calculated using baseline value for each corresponding bout. In bout 1 there was an attenuation of force production over time in the control group but not from PCM treatment ($P = 0.002$). This difference in force production between treatments was not evident for bout 2 ($P = 0.172$). Consistent with a RBE, force production was attenuated in the initial bout versus the repeated bout, but this effect was different between treatments (treatment by bout by time # $P = 0.002$).

7.3.2 Soreness

Overall soreness was lower in the PCM treatment group than in the control group ($F = 4.5$, $P = 0.044$, $ES = 0.87$; PCM CI: 0.9-2.0, control CI: 1.7-2.7). In bout 1 quadriceps muscle soreness showed a main effect of time ($F = 77.6$, $P < 0.0001$, $ES = 3.60$; Figure 23). In bout 1 overall recovery of soreness was independent of treatment ($P = 0.098$); however over time soreness recovered faster in the PCM treatment group than in the control group ($F = 4.1$, $P = 0.009$, $ES = 0.83$; PCM CI: 1.4-2.7, control CI: 2.1-3.4), indicating an attenuated recovery of soreness in the PCM treatment group. Although there was also a main time effect for soreness in bout 2 ($F = 31.6$, $P < 0.0001$, $ES = 2.29$), there was no difference in between treatments, as evident by the non-significant group ($P = 0.070$) and interaction ($P = 0.061$) effects. Consistent with a RBE soreness was overall lower in bout 2 than in bout 1 ($F = 26.2$, $P < 0.001$, $ES = 1.32$; B1 CI: 2.0-2.8, B2 CI: 0.8-1.7).

7.3.3 Skin Temperature

There was an overall treatment effect for skin temperature ($F = 118.9$, $P < 0.0001$, $ES = 5.44$; PCM CI: 27.4-29.0°C, control CI: 32.9-34.3°C). In the first bout, PCM treatment resulted in an average skin temperature of $23.9 \pm 0.7^\circ\text{C}$ for the 6-hour treatment duration compared with $33.9 \pm 0.9^\circ\text{C}$ during the control condition. Skin temperature did not differ between treatments in the second bout ($P = 0.264$).

7.3.4 Compression Pressure

Compression pressure was not different between treatments or across bouts in both seated ($P = 0.621$) or standing ($P = 0.209$) positions. Average compression pressure was 14.5 ± 1.7 mm Hg while seated and 8.7 ± 1.4 mm Hg while standing, which is negligible compared with the pressure needed to influence recovery through compression garments (14.8 ± 2.2 mm Hg while standing; Hill et al., 2017).

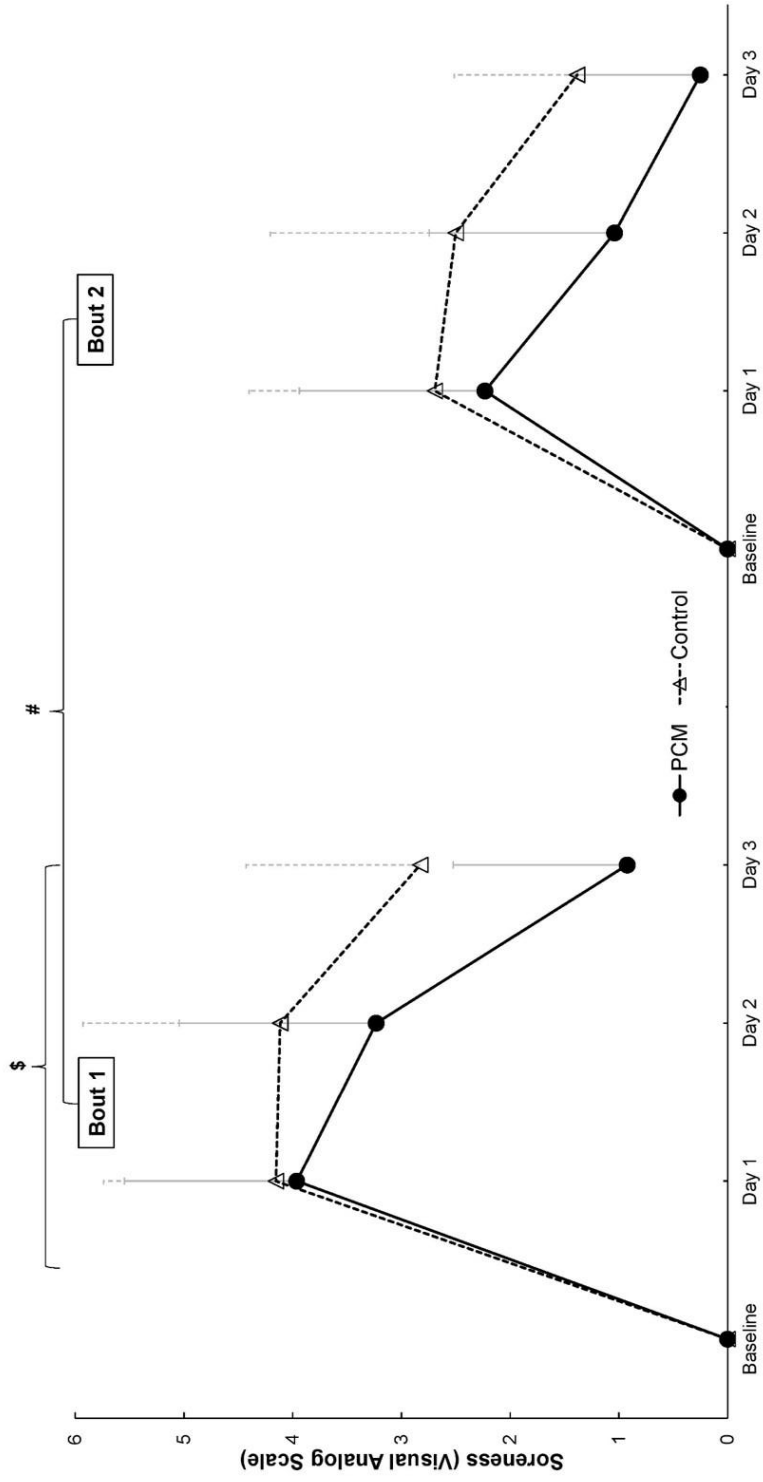


Figure 23: Subjective reports of quadriceps soreness on a 0-10 scale (0 = no discomfort, 10 = too painful to squat to 90°) for the PCM treatment and control groups before and on the three days following each bout of eccentric exercise. Values are mean \pm SD. Soreness was reduced in the repeated bout vs. the initial bout ($\#P < 0.001$) and this effect was not different between treatments ($P = 0.302$). However, magnitude of soreness was overall lower from PCM than control ($P = 0.044$). Soreness was lower over time in the PCM cooling group in bout 1 ($\$P = 0.009$). This difference was not evident in bout 2 ($P = 0.061$).

7.3.5 Blood Markers

Both the initial (time effect: $F = 4.6$, $P = 0.031$, $ES = 0.88$) and repeated bouts (time effect: $F = 17.3$, $P < 0.0001$, $ES = 1.70$) caused elevations in CK (% baseline; Figure 24a), with no difference between treatments or treatments over time in either bout. Despite % baseline CK showing a trend towards a bout effect, this was not significant ($P = 0.067$). Across the two bouts of exercise lnCK was overall lower for the PCM treatment group than for control (PCM: 2.25 vs control: 2.44; $F = 5.1$, $P = 0.033$, $ES = 0.92$; PCM CI: 2.14-2.37, control CI: 2.32-2.56). In bout 1, lnCK (log transformed) was elevated over time (Figure 24b; $F = 21.2$, $P < 0.0001$, $ES = 1.88$), and was overall higher in the control group vs the PCM treatment group ($F = 5.3$, $P = 0.031$, $ES = 0.94$; PCM CI: 2.09-2.35, control CI: 2.30-2.57) with no difference in elevation between treatments over time ($P = 0.896$). Although there was also a main time effect for lnCK in bout 2 ($F = 17.1$, $P < 0.0001$, $ES = 1.69$), there was no difference between treatments (treatment effect: $P = 0.160$) or treatment groups over time (treatment by time effect: $P = 0.099$). The overall lnCK response was comparable across both bouts (bout effect: $P = 0.516$) and was not different across treatments (bout by treatment effect: $P = 0.632$).

Overall, hsCRP was unaffected by the exercise stimulus regardless of bout (Table 10), with no difference between bouts ($P = 0.358$) and no difference between treatments (treatment effect: $P = 0.376$, bout by treatment effect: $P = 0.447$).

Table 10: Inflammatory indices response (hsCRP) in the blood before (baseline) and for 3 days (Day 1, 2, and 3) after the eccentric exercise for each bout of exercise in the PCM and control groups.

	PCM (ug/ml)		Control (ug/ml)	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline	0.66±0.48	0.73±0.57	0.76±0.48	0.86±0.57
Day 1	0.80±0.52	0.80±0.83	0.87±0.52	1.24±0.83
Day 2	0.71±0.57	0.98±0.72	0.83±0.57	1.19±0.72
Day 3	0.76±0.55	0.86±0.70	0.81±0.55	1.11±0.70
<i>Time Effect</i>	$P = 0.531$	$P = 0.324$	$P = 0.498$	$P = 0.175$

Note: Values are mean ± SD.

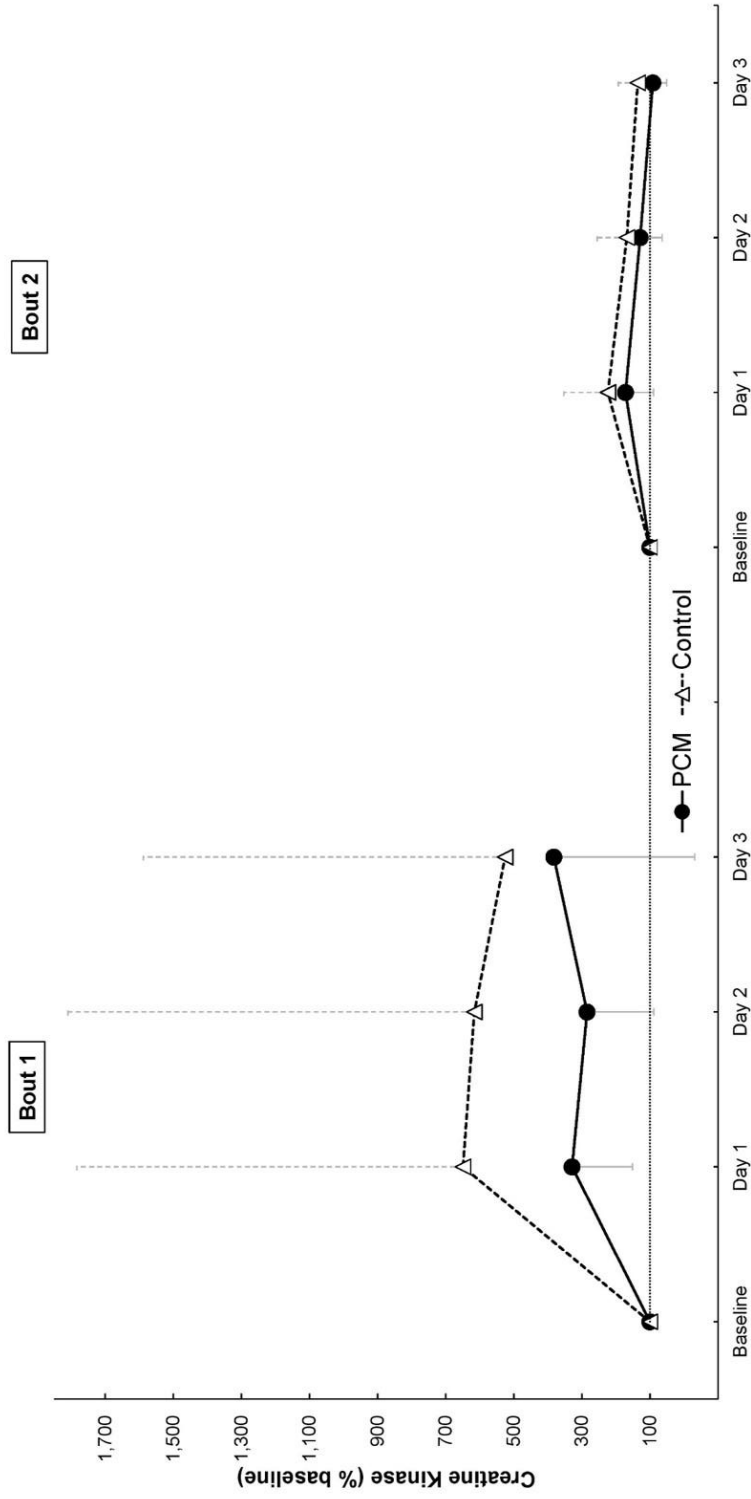


Figure 24a: Plasma CK (% baseline) before and following bout 1 and bout 2. Values are mean \pm SD. Both the initial ($P = 0.031$) and repeated bouts ($P < 0.0001$) caused elevations in CK. Despite there being a trend towards a bout effect ($P = 0.067$), the CK response was not different across bouts with no difference between treatments or treatments over time in either bout.

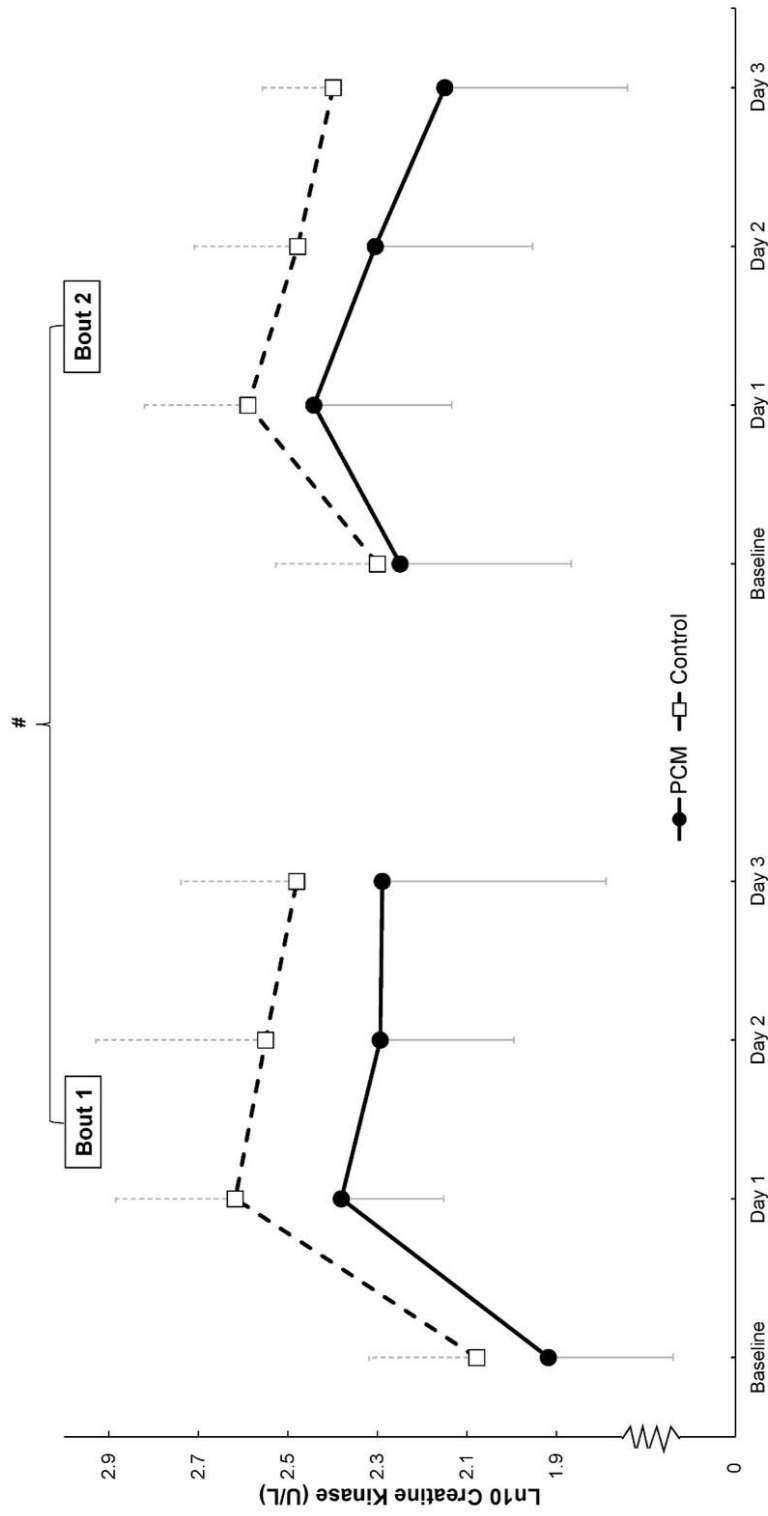


Figure 24b: Plasma CK activity for both PCM treatment and control groups before and following eccentric exercise bout 1 and 2. Values are mean \pm SD. lnCK was overall lower for the PCM treatment group than for control across the two bouts of exercise (#P = 0.033). In bout 1, lnCK was elevated over time (P < 0.0001), and was higher in the control group vs the PCM treatment group (P = 0.031). lnCK was elevated over time in bout 2 (P < 0.0001) but there was no difference between treatments or treatment groups over time.

7.4 Discussion

This study investigated whether protection provided by PCM cooling after an initial bout of eccentric exercise compromised the RBE. The exercise was successful in inducing muscle damage in bout 1, which was evident by the strength loss one day following the exercise in the control group (Figure 22). In the initial bout, PCM cooling accelerated recovery of strength and soreness vs the control group with no difference in CK elevation and minimal hsCRP elevation in either group. After the repeated bout there was no difference in strength, soreness, CK or hsCRP between groups, indicating that the protection provided by PCM cooling in the initial bout was not compromised when cooling was not provided after the repeated bout. This is the first investigation to examine the effect of prolonged cooling from PCM on the RBE, and the first investigation to demonstrate that PCM did not compromise the adaptive response associated with the RBE in bout 2. The findings following the exercise in bout 1, that PCM cooling augmented quadriceps muscle strength and accelerated recovery of soreness compared to the control condition by day 3, support the findings of chapter 4 which demonstrated PCM to be an effective strategy when recovering from EIMD of mechanical nature performed by untrained individuals.

Athletes have adopted CWI as a popular recovery modality aimed at limiting muscle damage and accelerating recovery (Versey et al., 2013). In a majority of the literature, single post-exercise CWI treatments have been used, with some studies administering CWI at intervals of 24 hours. Unlike the results of the present study and of chapter 4, the literature has failed to exhibit a favourable effect of CWI on the recovery of muscle strength using single immersion protocols (Leeder et al., 2012). When multiple immersions were administered within the first 24 hours following lower body exercise, CWI was effective in accelerating recovery of muscle strength compared to control (Skurvydas et al., 2006). Repeat immersions within the first 24 hours are logical, considering that exacerbation of muscle injury in the post-exercise period occurs in several stages (Paulsen et al., 2010); parts of which do not initiate until 2-6 hours following exercise (Armstrong et al., 1991). Unfortunately, multiple immersion sessions within this window may be inconvenient for athletes. Especially considering that access to CWI equipment over multiple sessions is difficult, and could mean that athletes need to remain at the training facility for long periods following competition or training. Prolonging the duration of cryotherapy application, with little logistical challenges, is an attractive benefit of PCM.

In addition to accelerating recovery of strength, PCM cooling also significantly reduced soreness compared to the control condition in bout 1. This was consistent with the results of chapters 4, as well as our research concurrent to this thesis applying PCM for 3 hours following a soccer match (Clifford et al., 2018). Importantly, although not significant, a trend was evident for reduced soreness from PCM cooling in bout 2 ($P = 0.061$). Thus, the findings of the present study imply that prolonged PCM cooling not only accelerated recovery of soreness following an initial bout of exercise, but this effect also did not inhibit the adaptive RBE response. Reduced soreness is also the most consistent effect of CWI (Bleakley et al., 2012; Hohenauer et al., 2015; Leeder et al., 2012; Machado et al., 2016). However, the overall effect of CWI on soreness has been proposed to be dependent on exercise mode, as well as the degree of muscle damage, or the training status of the individual. Both Leeder et al. (2011) and Ihsan et al. (2016) have suggested that CWI seems to be more effective in ameliorating effects of EIMD, such as soreness, induced by whole-body prolonged endurance exercise, intermittent based exercise, or high-intensity exercise, than following eccentric exercise. Although this was not the case as evident in chapter 5 of this thesis, prolonged PCM cooling was effective in accelerating recovery of soreness following a single exercise bout (chapter 4) as well as following the repeat exercise bouts in the present study.

This was the first study to measure CK following prolonged PCM cooling applied after eccentric exercise. There was a clear elevation in CK in bout 1 with no effect of PCM cooling and a clear RBE that was not different between treatments (Figure 24). Studies have previously demonstrated an RBE for CK (Howatson et al., 2007; Nosaka, Sakamoto, Newton, & Sacco, 2001). The lack of effect from PCM cooling on CK elevation in bout 1 was consistent with CWI literature showing no effect of CWI on CK efflux on the days after exercise (Anderson et al., 2018; Bailey et al., 2007; Bleakley & Davison, 2009; Fonseca et al., 2016; Halson et al., 2008; Howatson et al., 2009b; Leeder et al., 2015; Pointon et al., 2011b; Rowsell et al., 2009). This was also the first study to measure CRP following PCM cooling applied after eccentric exercise. Unfortunately, the exercise stress was insufficient to elevate CRP. Based on previous work, it was anticipated that performing bilateral eccentric quadriceps exercise at a high intensity would sufficiently elevate CRP (Margaritelis et al., 2015; Michailidis et al., 2013; Paulsen et al., 2005). However, compared to the present study which involved 120 eccentric contractions of each leg in the exercise protocol, the studies mentioned above performed 300 eccentric contractions (Michailidis et al., 2013; Paulsen et al., 2005), or 70 eccentric contractions repeated over multiple days

(Margaritelis et al., 2015) at various ranges of motion. The exercise protocol in the present study was perhaps insufficient in inducing myofibrillar disruption that would result in inflammation. Other studies have shown no elevation in CRP from similar eccentric protocols (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011). Studies using CRP to quantify the inflammatory response following CWI have also reported no effect (Banfi et al., 2007; Halson et al., 2008; Ingram et al., 2009)

This study was not without limitations. Primarily, the recovery of force to values above baseline in the PCM treatment condition in bout 1 indicates a potential learning effect. However, neither the isometric strength net torque values at baseline nor the average peak torque of the eccentric exercise were different between groups. Further as the same experienced investigator performed each MIVC whilst giving good instruction and stabilization and adhering strictly to the standardized procedure, it seems unlikely that only the treatment group experienced a learning effect. Thus, the results are a true treatment effect and comparable to the recovery of strength evident in chapter 4 of this thesis. Although the participants performed the eccentric exercise at 101% of their MIVC at 80° of knee flexion, the muscle damage response in bout 1 was smaller than anticipated. The lower limbs are less susceptible to EIMD than the limbs of the upper extremity (Saka et al., 2009), because the lower limbs are regularly exposed to prior bouts of eccentric exercise during day-to-day locomotion. The literature primarily utilises an upper extremity model to study EIMD (Clarkson & Tremblay, 1988; Newham, 1988; Nosaka, Clarkson, McGuiggin, & Byrne, 1991; Nosaka & Newton, 2002b). The upper limb muscle groups are less accustomed to eccentric loading and for this reason are more susceptible to damage but lack specificity when making inference to many sports and exercise (Howatson & van Someren, 2008). In the present study, participants performed eccentric contractions from 40-100° knee flexion to maximise the damage response. Strength loss and soreness have been previously demonstrated performing the same intensity isokinetic eccentric exercise from 70-110° knee flexion (McHugh & Pasiakos, 2004). By asking participants to reach a target torque of 90% MIVC during the eccentric exercise protocol in the present study, it transpired that participants exceeded the target torque and reached 100% of their MIVC, or greater. In bout 2, although the participants in the control condition exceeded 100% of their MIVC torque, performing the eccentric exercise at a greater intensity did not confound the results, as there was a RBE for all variables on the days following exercise. Further, participants hit the target torque at the lower spectrum of the knee flexion range of motion and therefore did not do much eccentric work at the longer muscle lengths. In retrospect, a range of motion between 70-110°

would have necessitated peak torque occurring at greater knee flexion causing more damage. Compounding this problem, participants were relatively well trained in the quadriceps (college ice hockey, and other sports requiring strong quadriceps). While these participants would have been susceptible to damage at long muscle lengths, they were likely protected against damage with the eccentric peak torque occurring at a shorter muscle length than intended.

Current evidence indicates that CWI is not universally beneficial for adaptation to exercise. In particular, regular repeat immersions during strength training attenuate muscle adaptations, and improvements in both maximal strength (Roberts, Raastad, et al., 2015; Yamane et al., 2015) and muscle mass (Fyfe et al., 2019; Roberts, Raastad, et al., 2015; Yamane et al., 2015). One of the proposed mechanisms behind these effects is an attenuation in the molecular mechanisms regulating resistance training adaptations (Broatch et al., 2018; Earp, Hatfield, Sherman, Lee, & Kraemer, 2019; Figueiredo et al., 2016; Lindsay, Carr, Othman, et al., 2015; Lindsay, Othman, Prebble, Davies, & Giesege, 2016; Roberts, Raastad, et al., 2015). However, athletes commonly perform dynamic exercises during training and competition, as well as participate in multiple exercise sessions over a season of sport, and sometimes even over 24 hours. In particular, in sporting events with multiple games occurring within short periods of each other, such as tournament play or fixture congestion, facilitating recovery is a priority over muscle adaptation. Hence, in the regular season of many sports, the goal of in-season management of the athlete is to facilitate recovery and avoid catabolism as opposed to inducing anabolic training adaptations. Thus, although the exercise paradigm utilized in the present study and in chapter 4 implemented an isolated exercise model which does not directly compare to the dynamic exercises most commonly performed by athletes, prolonged PCM cooling might still be efficacious in accelerating recovery following dynamic or team sport exercise as was evident in concurrent work related to this thesis (Brownstein et al., 2019; Clifford et al., 2018). Specifically, prolonged PCM cooling could be useful during periods of training when the exercise stress is higher than normal or when there is inadequate time for recovery, without concern of a detrimental effect on the adaptive response.

7.5 Conclusion

The only study to date examining the effects of CWI following exercise on the RBE failed to show any favourable effect on variables of EIMD following the initial bout of exercise (Howatson et al., 2009b). Thus, both the CWI and control groups exhibited

a RBE that was not compromised by the CWI. In contrast, following the initial bout of exercise in the present study, recovery of strength and soreness were accelerated from prolonged PCM cooling. Importantly, the adaptive response was not compromised by the prolonged cooling. Although the findings of this chapter and of chapter 4 established the efficacy of PCM to expedite recovery following an isolated exercise resulting in EIMD, these results can not be directly translated to a competitive athletic environment involving dynamic whole body movements and multiple bouts of exercise. However, implementing a full body exercise stress, comparable to an athletes training or competition regimen, was beyond the scope of this investigation. Thus, the present study supports the use of prolonged PCM cooling for its ability to mitigate decrements of strength and soreness following isolated exercise of mechanical nature while not interfering with the adaptive RBE response. The present study adds to evidence indicating that prolonged PCM cooling following eccentric exercise could enhance both short- and longer-term recovery of strength and soreness. The precise mechanisms for accelerated recovery from prolonged PCM cooling remain to be elucidated.

7.6 Perspectives

Since the overall aim of this thesis is to elucidate the effects of PCM on recovery from exercise, it was pertinent to establish these effects not only following a single exercise session but also on subsequent exercise bouts. Especially since the RBE is a naturally occurring adaptive response. The previous chapters implemented prolonged PCM cooling following a single bout of exercise. It is well understood that a second bout of comparable eccentric exercise reduces the signs and symptoms of EIMD. Thus, this chapter investigated the influence prolonged PCM cooling had on the RBE. It was possible that if PCM cooling blunted the inflammatory response in the first bout of exercise, the RBE would not have been evident in the second bout in the PCM treatment group. This would have indicated that PCM cooling had a detrimental effect on the normal RBE. However, this response was not the case, as evident by the lack of strength loss and lower soreness in the PCM group in bout two of the present chapter.

The findings of the present study support the findings from chapter 4 by demonstrating that PCM cooling enhances the recovery of strength and reduces soreness following a single bout of eccentric exercise. Further, this study provides new information about the effect, or lack thereof, of prolonged PCM cooling on blood markers of muscle damage following eccentric exercise, which were not measured in

chapter 4. Although PCM cooling did not affect the CK response in bout 1 or the hsCRP response in both bouts, following the second bout of exercise CK recovered more rapidly in the group receiving PCM treatment. Importantly, these data show for the first time that prolonged PCM cooling does not compromise the adaptive response associated with the RBE. The findings of this chapter, coupled with the results of the previous chapters, address the overall aim of the thesis by indicating that prolonged PCM cooling can be utilised by athletes to accelerate recovery over repeated bouts of eccentric exercise without hindering the adaptive response.

8.0 GENERAL DISCUSSION AND FUTURE DIRECTIONS

8.1 Chapter Summary

Interventions that attempt to attenuate the signs and symptoms of EIMD and accelerate recovery following exercise have become popular among athletes in order to gain a competitive advantage. Cryotherapy, the application of cold for therapeutic purposes, has recently gained popularity. The most popular form of cryotherapy, CWI, has received a great deal of attention in the literature. Despite its growing popularity, there remains conflicting evidence to support the use of CWI for recovery from exercise. Most commonly, CWI is reported to be beneficial for reducing DOMS (Bleakley et al., 2012; Leeder et al., 2012); and its effects on accelerating recovery of other variables are negligible (Poppendieck et al., 2013). Furthermore, there is some evidence suggesting that the therapeutic effects attributed to cryotherapy treatment might be due to a placebo effect (Broatch et al., 2014). Several factors could explain the overall lack of effect of CWI, and other cryotherapy modalities, on recovery from exercise. These include variability in the exercise model, immersion temperature, duration, and frequency. Ultimately, the magnitude of change in tissue temperature has been positively correlated with cryotherapy methods of longer duration (Peiffer, Abbiss, Watson, et al., 2009). Consequently, the overall aim of this thesis was to investigate and establish the efficacy of a novel cryotherapy modality (PCM) capable of prolonging the cooling duration on accelerating recovery from exercise (see figure 25).

The first experimental chapter of this thesis was a pilot study aiming to establish the efficacy of PCM cooling as an alternative cryotherapy intervention for recovery of strength and soreness in the quadriceps on the days following bilateral isolated eccentric exercise. The results from the initial study served as a proof of concept by establishing that prolonged PCM cooling accelerated recovery of strength and soreness following muscle damage of mechanical nature in a single muscle group. Since there were three treatment groups: direct cooling, indirect cooling, and control; and the treatment effects for strength and soreness were not different between the direct and indirect cooling conditions, the results indicated that a systemic effect might have occurred in the indirect cooling leg from the leg that received direct cooling. This study also demonstrated the practical utility of a wearable garment to deliver prolonged cooling to muscles after damaging exercise, and suggested that PCM worn for 6 hours was well tolerated.

The second chapter implemented a metabolic stress exercise model in order to determine the efficacy of PCM cooling for accelerating recovery following a marathon

run. In addition to measuring strength and soreness, which were also measured in the pilot study, this investigation also measured CMJ height and blood markers of muscle damage (CK) and inflammation (hsCRP). The marathon led to decreases in muscle function, increases in perceptions of soreness, and increases in blood markers of muscle damage and inflammation. Despite the positive effects from prolonged PCM cooling evident in the pilot study, the application of 3 hours of PCM cooling did not offer an advantage for accelerating recovery of any of the variables following the marathon run. Overall, these results suggested that PCM cooling is not an effective recovery modality for exercise encompassing a large metabolic component.

The third chapter determined the degree of cooling occurring at the skin, in the muscle, and in the body while 3 hours of PCM cooling were applied to the quadriceps. These effects were also measured in a 15-minute temperature matched (15°C) CWI treatment. The cardiovascular response to both cryotherapy modalities was also determined. In this investigation, the magnitude of temperature reduction from PCM was comparable to CWI, but the intramuscular temperature was decreased for longer for the duration of PCM cooling. Additionally, a central effect was confirmed by the reduction in core temperature and the increase in HRV, during both treatments. These findings provide evidence that the effects at the musculature from PCM cooling are comparable to those from CWI treatment and explain in part the accelerated recovery from PCM cooling in the pilot study.

The final investigation was designed to build on the findings of the pilot study in a larger sample of participants, with a between-groups design to negate the potential confounding effect of a systemic effect. The exercise protocol was repeated by all participants two weeks later in order to establish the effects of prolonged PCM cooling on the adaptive response to exercise. Additionally, the blood markers that were measured in chapter 5 were also measured in this study. Unlike the marathon run in chapter 5, the results indicated that the isolated eccentric exercise did not increase the markers of muscle damage and inflammation. However, the results supported the findings of chapter 4, as the recovery of strength and soreness were again accelerated, while also demonstrating that prolonged cooling did not interfere with the RBE.

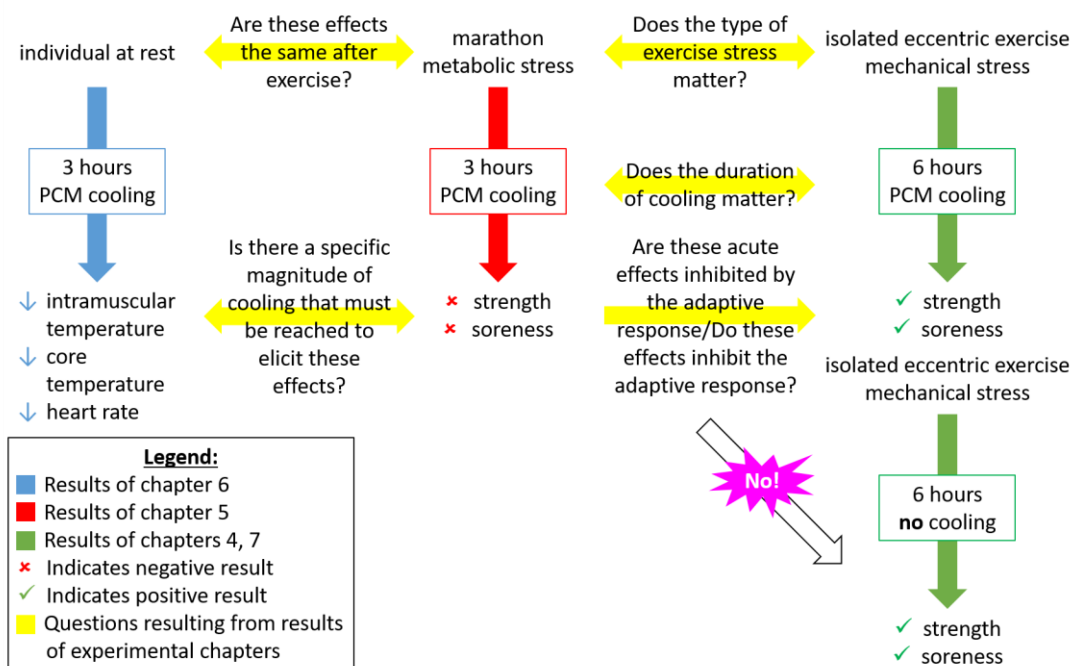


Figure 25: Schematic illustration of the main findings establishing the efficacy of a novel cryotherapy modality (PCM) capable of prolonging the cooling duration on accelerating recovery from exercise. Blue arrow illustrates the physiological effects of PCM cooling in an unexercised thermoneutral individual (chapter 6). Red arrow illustrates the effects of PCM cooling in an individual following metabolic stress, a marathon run (chapter 5). Green arrow illustrates the effects of PCM cooling in an individual following one bout of mechanical stress, isolated quadriceps eccentric exercise (chapter 5), and the effects from PCM cooling following a repeated bout of the same exercise (chapter 7). The yellow arrows indicate questions arising from the results presented throughout the chapters encompassing this thesis. The question of whether the beneficial effects from PCM cooling on accelerating recovery of strength and soreness following one bout of isolated eccentric exercise was answered in chapter 7.

8.2 Discussion

Athletes have been turning to a wide range of recovery modalities in order to alleviate the signs and symptoms of EIMD. A modality capable of reducing the severity of muscle damage and lessening the strength loss and soreness that are a common consequence resulting from EIMD would accelerate the recovery period. The results

from the investigations encompassing this thesis provide novel findings that ultimately may give athletes a competitive advantage over their peers, if implemented appropriately. The chapters included within this thesis exploited the ability of a novel cryotherapy modality, PCM frozen at 15°C, to deliver a single dose of prolonged cooling. In humans, prolonging the duration of cooling has previously only been achieved by repeating cryotherapy treatments. Extending the positive effects associated with cryotherapy, based from data evident in animal models (see section 2.2.2), may translate to greater recovery following exercise; but to date results in humans are scarce and inconclusive.

The findings of this thesis suggest that prolonging the duration of cooling successfully accelerates recovery of strength and soreness following isolated eccentric exercise (chapter 4), and that this effect does not inhibit the RBE (chapter 7); but that prolonged cooling does not elicit comparable effects following a marathon run (chapter 5). These results generally concur with the existing body of literature that supports the use of CWI for accelerating subjective measures of soreness following exercise (see section 2.2.5.3.2). However, the findings from this series of investigations have led to some novel conclusions. Primarily that, unlike CWI which has generally failed to show any benefit on the recovery of strength (see section 2.2.5.3.3), prolonged PCM cooling was capable of successfully accelerating recovery of strength following eccentric exercise and that this acute effect did not hinder the adaptive response to exercise when the exercise was repeated. Furthermore, the investigations demonstrated that prolonged PCM cooling was effective in accelerating recovery following eccentric exercise but not following marathon running. Although investigating the mechanism behind why prolonged PCM cooling accelerated recovery following mechanical stress but not metabolic stress were beyond the scope of this thesis, this finding led to some interesting speculations. Secondly, the results of chapter 6 provided data to support the physiological cooling efficacy of the prolonged duration of PCM whilst contrasting these effects with a temperature matched CWI protocol. The results from chapter 6 additionally presented the novel effects from local prolonged PCM cooling vs systemic short duration CWI on the autonomic response in individuals at rest, and surprisingly concluded that local PCM cooling successfully affected the neural activity of the heart.

Whilst this PhD has identified the ability for PCM cooling to accelerate recovery of strength loss and soreness following isolated eccentric exercise, the finding that prolonged PCM cooling was ineffective in accelerating recovery following marathon running was surprising. The finding that PCM cooling was unsuccessful in

accelerating recovery following exercise encompassing a large metabolic component but not following exercise encompassing a mechanical component, is contradictory to previous reviews. Previously, Ihsan et al. (2016) and Leeder et al. (2011) both concluded that CWI is more effective in alleviating symptoms of EIMD following exercise with a high metabolic cost, such as endurance, high intensity, or team sport exercise, but not following eccentric exercise. Collectively these reviews suggest that CWI is more effective for recovery from metabolically stressful exercise, as opposed to mechanically stressful exercise. Furthermore, in their review, White & Wells (2013) proposed that CWI may be especially beneficial for recovery from metabolically stressful exercise because of its ability to restore cardiovascular function. However, when utilising PCM as the cryotherapy modality, the results from the chapters encompassing this thesis oppose the aforementioned findings.

Although exercise associated with either mechanical or metabolic stresses results in similar events including increased cytosolic Ca^{2+} , sarcolemmal permeability, muscle fibre oedema, disruption to cell structures and resultant soreness and strength loss, there is a difference in the aetiology and extent of muscle fibre damage, temporal sequence of events and magnitude of the inflammatory response (White & Wells, 2013; see section 2.1.1 for review). Mechanical stress imposed on the skeletal muscle during exercise results in direct physical disruption of the sarcolemma, sarcomeres, and the E-C coupling system within the muscle (Armstrong, Ogilvie, & Schwane, 1983). Ultimately, muscle strength is proposed to be reduced due to disrupted sarcomeres within the myofibrils and damage to the E-C coupling system (Armstrong et al., 1991; Proske & Allen, 2005; Warren, Ingalls, Lowe, & Armstrong, 2001). On the other hand, physical disruption occurs indirectly as a result of metabolic deficiencies following exercise with a large metabolic component such as marathon running, and a significant central fatigue component contributes to the impairments in strength (Millet et al., 2003; Minett & Duffield, 2014). Indeed, Skurvydas et al. (2016) proposed different mechanisms that influence the recovery of strength following mechanical vs metabolic stress. Namely, that mechanically demanding contractions result in prolonged reductions in strength due to myofibrillar loss of integrity; while strength loss following metabolically demanding exercise is likely due to reduced Ca^{2+} release at the sarcoplasmic reticulum and/or myofibrillar Ca^{2+} sensitivity. Since PCM was effective for accelerating recovery from EIMD induced through mechanical stress throughout this thesis, but not metabolic stress, it is possible that PCM cooling ameliorated the magnitude of structural disruption associated with mechanical stress. It is further possible that a beneficial effect from

PCM cooling following metabolically stressful exercise, such as a marathon, might only become evident once metabolic activity within the muscle is sufficiently reduced. Therefore, understanding that there are different mechanisms involved in the processes associated with mechanically vs metabolically induced EIMD, and that there are likely several mechanisms involved in the cryotherapy mediated recovery from exercise, it is reasonable to believe that cryotherapy could facilitate recovery from metabolically stressful exercise differently than from mechanically stressful exercise.

Although the findings of this thesis suggested that the exercise modality used to induce EIMD seems to influence the effectiveness of PCM in facilitating recovery, it was beyond the scope of research to determine the physiological mechanisms behind this effect. Following exercise, the inflammatory response involves the mobilisation of neutrophils and lymphocytes to the damaged muscle tissue via soluble intercellular adhesion molecule 1, and a production of pro-inflammatory cytokines in muscle (Clarkson & Hubal, 2002; Peake et al., 2005; Paulsen et al., 2012). Together, these substances cause intramuscular degradation, which amplifies the initial muscle damage (Clarkson & Hubal, 2002; Peake et al., 2005; Paulsen et al., 2012). In theory, following EIMD, the physiological responses to cold exposure whereby vasoconstriction reduces the permeability of blood vessels to immune cells and reduces the exposure of the peripheral tissues to inflammatory cells (Hauswirth et al., 2011; Lee et al., 2005; Pournot et al., 2011; Ferreira-Junior et al., 2014) should attenuate the acute inflammatory process and enhance recovery by protecting the muscle from secondary muscle damage. An attenuated secondary damage response was indirectly evident by the accelerated recovery of strength following the isolated eccentric exercise in chapters 4 and 7. However, chapter 7 indicated that the isolated eccentric exercise was insufficiently damaging to cause elevations in systemic inflammation as measured using hsCRP throughout this thesis. This finding is not surprising as humans can experience symptoms of EIMD without presenting signs of inflammation (i.e., leucocyte infiltration) in the muscle tissue (Malm et al., 2000; Paulsen et al., 2010, 2010, 2012; Raastad, Risoy, Benestad, Fjeld, & Hallen, 2003). Ultimately, since the exercise stimulus in chapters 4 and 7 was not stressful enough to increase inflammation, it is difficult to elucidate what effect, if any, PCM cooling has on the inflammatory response following eccentric exercise. On the contrary, the marathon in chapter 5 resulted in both strength loss and an elevated hsCRP response. It is commonly assumed that cryotherapy accelerates recovery from muscle damage by decreasing the inflammatory process. If cryotherapy were to

decrease the pro-inflammatory response, causing fewer neutrophils and lymphocytes to transmigrate into the damaged muscle tissue, this effect would be reflected by a decrease in hsCRP. However, the mechanisms behind this effect are largely unknown and the ability of cryotherapy to accelerate recovery may likely be attributed to mechanisms other than a reduction in inflammation, such as the magnitude of tissue cooling itself.

The rate of post-exercise recovery is related to the extent of the load imposed on the various physiological and neuromuscular systems by the exercise bout (Nédélec et al., 2012). Since marathon running involves multiple muscle groups, is associated with systemic hyperthermia, and a large central fatigue component, the physiological demands of the marathon run in chapter 6 were likely far greater than the isolated eccentric exercise in chapters 4 and 7. It is then likely that the differing physiological demands of the two exercise types contributed to the disparity in the ability of prolonged local PCM cooling to accelerate recovery following eccentric exercise, but not following a marathon run. Therefore, the PCM mediated recovery was likely dependant on the cryotherapy dose being large enough to successfully mediate the magnitude of damage resulting from metabolic stress. Since the magnitude of damage was much greater following the marathon run compared to the eccentric exercise, as evident by the elevated inflammatory response in chapter 5 but not chapter 7, it is possible that the magnitude of local PCM cooling was insufficient following the marathon but sufficient following the eccentric exercise. As an example, previous studies that have successfully implemented CWI for the accelerated recovery of strength have all done so following exercise in the heat, which results in increased thermal strain and central fatigue (Ihsan et al. 2016; Minett et al., 2014; Pointon et al., 2011b). In the aforementioned studies, recovery of strength was concomitant with the amelioration of voluntary activation and core temperature. Therefore, under a large thermal load, CWI may alleviate some of the exercise-induced cerebral perturbations either directly or via its effect on core temperature (Ihsan et al., 2016). Therefore, since the isolated eccentric exercise performed in chapters 4 and 7 likely did not induce substantial thermal strain or central fatigue, the magnitude of PCM cooling was sufficient to ameliorate the secondary effects associated with the EIMD processes. On the contrary, since PCM was only applied locally to the quadriceps following the marathon in chapter 5, it is possible that the degree of cooling might have been insufficient to ameliorate the damaging mechanisms occurring as a result of the substantial amount of metabolic stress.

PCM cooling differs from all other cryotherapy modalities in its ability to deliver cooling for a prolonged duration. Clearly the dose of PCM cooling within the chapters encompassing this thesis was sufficient to influence recovery following the isolated eccentric exercise but not following the marathon run. However, the total duration of PCM cooling was 6 hours following the eccentric exercise in chapters 4 and 7, but only 3 hours following the marathon run in chapter 5. Although core temperature was not measured during the exercises in the aforementioned chapters, endurance type protocols characteristically result in a greater level of systemic hyperthermia and an elevated thermal load compared with isolated exercise such as resistance exercise (Deschenes et al., 1998; Mortensen et al., 2008). Core temperature was however measured in chapter 6 of this thesis. Chapter 6 demonstrated that local PCM application was capable of reducing core temperature and restoring autonomic balance in individuals in a rested state. Although not directly investigated in this thesis, it is likely that a comparable response would not have been evident from the local PCM cooling, particularly not in the individuals having completed a marathon run. Although the local PCM cooling in chapter 6 provided enough of a thermal stimulus to influence convective cooling which decreased core temperature, it is likely that convective cooling from local PCM application would not occur in individuals under a significant thermal load. Specifically in the individuals who completed a marathon run it is likely that an insufficient surface area was exposed to the cold stimulus at the periphery, limiting the exposure of the blood to the cold tissues, and thus inhibiting a change in the core temperature. For this reason CWI, which exposes a larger surface area of the body to the cold stimulus resulting in greater temperature gradients for tissue cooling post-exercise, should overcome this issue. However, Wislon et al. (2018) have previously unsuccessfully administered CWI as a recovery strategy following a marathon run. The results of chapter 6 indicated that CWI can induce a rapid drop in muscle and core temperatures while PCM cooling provides a gradual prolonged decrease in temperatures. Therefore, if the goal is to maximise the tolerable decline in peripheral muscle temperature whilst simultaneously delivering a cooling stimulus capable of eliciting central effects on core temperature for a sustained duration, athletes and practitioners might opt to combine the two treatments. Particularly if the exercise stimulus was likely to have increased the individuals thermal load. In practice, once an athlete completed a CWI treatment, quickly decreasing their intramuscular and core temperature, they could apply PCM over muscle groups they wish to keep cool in order to maintain the reduction of both peripheral and central temperatures. This could allow the athlete to sustain the treatment effect from CWI for a longer duration in the immediate post-exercise period.

All the while allowing the athlete to return to normal post-exercise activities (e.g. meal, relaxation, recreational activities) while receiving a cryotherapy dose.

Alternatively, animal models have previously shown that a window of opportunity for intervention with cryotherapy lies within the first 30 minutes after injury (Merrick & McBrier, 2010). Therefore, a decrease in the muscle temperature during this period can prevent the exacerbation of the secondary damage response (Merrick et al., 1999). Reducing muscle temperature and creating a local hypothermic environment during the window of opportunity may decrease the metabolic activity within the muscle. Ultimately, reducing muscle metabolism would prevent excessive Ca^{2+} excretion into the cytosol, which would inhibit glycolysis and the degradation of ATP by myofibrillar adenosine triphosphatase. This effect would be particularly efficacious for recovery following endurance exercise because it would inhibit the disrupted Ca^{2+} homeostasis that results in strength loss following metabolically demanding exercise. Unfortunately reductions in muscle temperature to the degree suggested to reduce muscle metabolism in animals (Sapega et al., 1988) have not been demonstrated in humans in the literature or in chapter 6 of this thesis. This issue was further compounded by the fact that the participants did not apply the PCM cooling until on average 80 minutes after exercise cessation following the marathon in chapter 5. On the contrary, the participants in chapters 4 and 7 received the PCM within 10 minutes of exercise cessation. Similarly, the PCM in Clifford's (2018) and Brownstein's (2019) investigations was administered within 45 and 30 minutes of cessation of exercise, respectively. The rapid deployment of PCM following the isolated eccentric exercise that took 40 minutes to complete in chapters 4 and 7 of this thesis and following the 90 minute soccer games (Brownstein et al., 2019; Clifford et al., 2018) was a likely variable leading to the success of PCM in accelerating recovery. Conversely, since the rate of secondary injury is slowly developing and long-lasting (> 5-hour duration; Merrick & McBrier, 2010), the delay in the application of PCM following the marathon meant that the PCM were likely applied too late into this process. Muscle damage is likely induced before the marathon run is concluded; thus, some prolonged duration of overlap exists between the exercise being performed and the muscle damage occurring. This overlap was likely greater during the marathon run than in the studies showing a beneficial effect from PCM, where the exercise took between 40-90 minutes to complete. Although not methodologically possible following the marathon in chapter 5 of this thesis, athletes, particularly those completing exercise of long durations, should make every effort to prioritise the application of cryotherapy as soon as possible upon cessation of exercise.

Perhaps most pertinent to athletic populations are the direct findings of chapter 7 of this thesis. Chapter 7 administered PCM cooling following isolated eccentric exercise and implemented a repeated bout of exercise in order to observe the effects of cooling induced accelerated recovery evident in chapter 4 on the RBE. Since the magnitude of the protective effect of the RBE is a naturally occurring adaptive mechanism, if the recovery modality blunted the inflammatory response following the first bout of exercise, the RBE might have been attenuated following the second bout of exercise. Repressing acute inflammation may negatively affect the repair and regenerative processes of skeletal muscle (Woods, Lu, & Lowder, 2000) and may be detrimental to muscle (Barnett, 2006; Yamane et al., 2005), and functional performance (Costello & Donnelly, 2011). Thus athletes may be concerned that inhibiting this response would put them at a training and performance disadvantage. Especially because repetitive CWI exposures have previously been shown to effect long-term adaptation to training by blunting chronic adaptations (Higgins et al., 2011; Yamane et al., 2005); such as attenuating muscular and vascular adaptations (Yamane, Ohnishi, & Matsumoto, 2015; Motoi Yamane et al., 2005), suppressing the cellular signalling required for adaptation (Lindsay, Othman, Prebble, Davies, & Giesege, 2016) and activation and deregulation of the inflammatory process (Lindsay et al., 2015). Furthermore, the literature suggests that chronic CWI exposure impedes hypertrophic/strength adaptations (Figueiredo et al., 2016; Fröhlich et al., 2014; Roberts, Raastad, et al., 2015; Yamane et al., 2015; Yamane et al., 2005). Although the PCM application in chapter 7 was not repeated following the second bout of exercise, this was done intentionally in order to clarify the effects of accelerated recovery on acute adaptation. Chapter 7 demonstrated that although PCM cooling attenuated the severity of strength loss and soreness following the first bout of exercise, the RBE was not inhibited in the PCM treatment group following bout 2. This finding indicated that PCM cooling does not inhibit the adaptive response to exercise. Therefore, athletes interested in implementing PCM cooling into part of their recovery practice may opt to forgo cryotherapy as a recovery modality during their pre-season, when building muscular strength is a priority. However, when maintaining performance is a priority over building muscle during the in-season, athletes should not be concerned about the acute effects of PCM cooling for accelerating recovery inhibiting the adaptive response between exercise sessions. Ultimately, the benefits of PCM for providing acute recovery could increase an athletes total time training or the overall training load in subsequent sessions during intense athlete schedules.

This thesis aimed to investigate the novel ability of PCM to prolong the cryotherapy dose on the efficacy of recovery from exercise. The results from this thesis could have a number of important practical implications and might indirectly inform the real-world utilisation of PCM for recovery by athletes during training and competition. Collectively the results presented throughout this thesis support previous findings successfully utilising CWI for reducing muscle soreness. Additionally, the results support for the first time the concept of prolonging the cooling period through the use of 15°C PCM to elicit beneficial effects on recovery of strength loss following eccentric exercise, and that a prolonged duration of cooling does not inhibit the RBE. Furthermore, the prolonged duration of cooling delivered by the PCM throughout the studies was well tolerated, and no adverse events were reported. For these reasons PCM might be a better cryotherapy alternative than CWI particularly in scenarios when interventions like CWI are logistically challenging to implement. Not only does PCM cooling allow for the dose of cooling to be prolonged, but PCM cooling provides athletes with the ability to continue with their activities of daily living while simultaneously receiving a cryotherapy dose. Ultimately PCM cooling at 15°C offers rapid and convenient deployment of a recovery strategy capable of accelerating the signs and symptoms associated with EIMD.

The series of investigations encompassing this thesis have culminated into 5 main conclusive points (see Figure 25): 1) six hours of PCM cooling is an efficacious strategy for accelerating recovery of strength and soreness following isolated eccentric exercise and 2) this effect does not inhibit the RBE, but 3) three hours of PCM cooling was not effective at attenuating the deleterious effects of EIMD following a marathon run, which suggests that 4) either the mechanism with which prolonged PCM cooling accelerates recovery differ between mechanically and metabolically induced EIMD, or the magnitude of cooling was sufficient following the eccentric exercise but not following the marathon, and finally 5) the evidence from chapter 6 suggests that in rested individuals the efficacy of 3 hours of PCM cooling on reducing intramuscular temperature, core temperature, and the cardiovascular response is comparable to that of CWI however these effects can not be directly translated to an exercise paradigm.

8.3 Limitations and Future Research Directions

While the series of investigations encompassing this thesis have produced a number of novel and interesting findings, they are not without limitations and have raised several questions for future research. Firstly the isolated eccentric exercise protocol

implemented in chapters 4 and 7 does not allow for the results to be directly translated to an athletes training or competition regimen. For example, although the PCM cooling was efficacious in accelerating recovery of strength and soreness in chapters 4 and 7, the exercise stress was isolated to a single muscle group. As was discussed in the previous section, full body exercise might elevate the thermal load to such a degree that the local cooling stimulus is no longer effective. On this note, in concurrent work related to this thesis (Brownstein et al., 2019; Clifford et al., 2018), PCM were applied following a soccer match which is an exercise encompassing both mechanical and metabolic stress components. In both cases recovery following the soccer match was accelerated in the groups receiving PCM treatment. This finding lends support towards the applicability of the positive results from chapters 4 and 7 of this thesis in all-encompassing athletic environments. However, when paired with the results of chapter 5 which showed no effect of PCM on accelerating recovery following a marathon, this finding also suggests that the dose or duration of PCM cooling should be tailored to the exercise type. Especially because exercise is heterogeneous and it would be erroneous to recommend a perennial PCM dose without accounting for the exercise type.

The variable efficacy of PCM cooling in accelerating recovery throughout the chapters of this thesis indicates that the magnitude of cooling is important. Although chapter 6 established the cooling efficacy of 3 hours of prolonged PCM cooling, and demonstrated that the magnitude of cooling was comparable to a temperature matched CWI protocol, the participants subjected to the cryotherapy treatments were in a rested state. It is highly likely that the magnitude of change in the muscle, and core temperature, and cardiovascular response evident in chapter 6 would be reduced in individuals having just completed exercise. Investigations examining the physiological response of PCM cooling following both mechanically and metabolically induced EIMD are necessary in order to elucidate whether the magnitude of the PCM cooling effect differs between these two conditions. Investigating these effects would also help answer what was the magnitude of cooling in the chapters implementing the eccentric exercises that allowed for the successful reduction in the damage response. Furthermore, investigating these effects whilst manipulating the amount of skin surface area exposed to PCM following a marathon would allow for conclusions to be made regarding the appropriate magnitude of cooling necessary in order to successfully influence recovery following metabolically stressful exercise.

Although beyond the scope of this investigation, recent research suggests that implementing the chronic use of cold modalities such as CWI can be detrimental to

training adaptations in athletes. Concern exists as to whether repetitive CWI exposure affects long-term adaptation to training by blunting chronic adaptations (Higgins et al., 2011; Yamane et al., 2005). Especially since some of the acute post-exercise effects purportedly blunted by CWI also stimulate exercise-induced adaptations (Broatch et al., 2018). Although the investigation in chapter 7 of this thesis only administered PCM cooling following the first bout of exercise, the results indicated that an acute dose of prolonged cooling was not detrimental to the adaptive response. Since the series of investigations encompassing this thesis administered a single session of PCM cooling, it would be of value to monitor the effects of chronic PCM treatment on recovery indices. Additionally, in the future, it would be worthwhile to determine whether chronic use of PCM has detrimental effects similar to those seen from CWI. However, since chronic CWI has demonstrated alterations in the response of tissue and core temperature, blood flow, inflammation and metabolism (Versey et al., 2013), it might be likely that the hypothetically smaller overall magnitude of cooling from local PCM application would prevent these alterations from occurring.

As with all cryotherapy modalities, when subsequent exercise is performed shortly after cooling, muscle temperatures may be unfavourably cold inhibiting subsequent performance (García-Manso et al., 2011; Halson et al., 2008; Versey et al., 2013; Wilcock, Cronin, & Hing, 2006b). A reduction in skin temperature by 10-13°C can promote a reduction of 10-33 % in nerve conduction velocity (Algaflly, George, & Herrington, 2007). Further, for every 1°C reduction in muscle temperature, a 3-6% reduction in contractile force has been demonstrated (Costello et al., 2012a; Sargeant, 1987). Muscle temperature reduced to suboptimal physiological levels (Algaflly & George, 2007; Costello et al., 2012a) are associated with decreased muscular contractile speed (Abramson, 1966; Bergh & Ekblom, 1979; Howard, Kraemer, Stanley, Armstrong, & Maresh, 1994; Rutkove, 2001), strength (Herrera, Sandoval, Camargo, & Salvini, 2010; Johnson & Leider, 1977; Rutkove, 2001; Yona, 1997), and altered neuromuscular properties (Dewhurst et al., 2009; Vieira, Oliveira, Costa, Herrera, & Salvini, 2013). Thus, local PCM cooling is not recommended in athletes performing high-intensity, explosive exercise unless an adequate warm-up is completed to increase internal body temperatures. On the contrary, when exercise is performed in warm environments, and/or when core temperature is elevated resulting in performance decrements, PCM between exercise bouts can be utilised to pre-cool prior to the subsequent exercise. In this scenario it would be most beneficial for PCM to be applied to the torso in the form of a cooling vest. Alternatively, PCM may have an application during the training component of exercise encompassing

primarily metabolic stress such as a marathon run. As was evident in chapter 5, the marathon run significantly increased systemic inflammation. If runners reach similar levels of inflammation during their training, PCM in between exercise bouts might be applicable as more of a recovery strategy in the lead up to the actual marathon itself. Prolonging the dose of cooling in between exercise sessions in these athletes might also restore vagal tone and normalise parasympathetic modulation of HR in between intense training sessions. On this note, more work is required to elucidate the mechanisms through which cryotherapy influences the inflammatory response following exercise.

9.0 APPENDICES

9.1 Appendix 1 Northumbria Informed Consent

Informed Consent Form

Project Title:

Principal Investigator: Susan Kwiecien

Investigator Contact Details:

Participant Number:

*please tick
where applicable*

I have read and understood the Participant Information Sheet.	<input type="checkbox"/>
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	<input type="checkbox"/>
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	<input type="checkbox"/>
I agree to take part in this study.	<input type="checkbox"/>
I would like to receive feedback on the overall results of the study at the email address given below. I understand that I will not receive individual feedback on my own performance.	<input type="checkbox"/>
Email address.....	

Signature of participant..... Date..... (NAME IN BLOCK LETTERS).....
Signature of Parent / Guardian in the case of a minor

Signature of researcher..... Date..... (NAME IN BLOCK LETTERS).....
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9.2 Appendix 2 Northwell Health Informed Consent

Informed Consent Summation/Participant Signature

You have read the above description of the research study. You have been told of the risks and benefits involved and all your questions have been answered to your satisfaction. A member of the research team will answer any future questions you may have. You voluntarily agree to join this study and know that you can withdraw from the study at any time without penalty. By signing this form, you have not given up any of your legal rights.

Printed Name of Participant	Participants Signature	Date
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Witness's Printed Name	Witness's Signature	Date
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(Note: A witness can be a member of the research team, but cannot be the same person signing consent as the investigator)

Investigator's Statement

In addition to advising the above participant of other forms of treatment and therapy which are appropriate, I have offered an opportunity for further explanation of the risks and discomforts which are, or may be associated with this study and to answer any further questions relating to it.

Investigator's signature	Date
--------------------------	------

Investigator's printed name

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