The impact of cryotherapy versus placebo interventions on recovery following strenuous exercise, and adaptations to resistance training

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Abstract

Strenuous exercise leads to muscle soreness, decrements in muscle function, and increases in circulating intracellular proteins and inflammatory markers. The magnitude of change and time course of recovery of these symptoms is largely dependent upon exercise mode. Cryotherapy, (cold water immersion (CWI) or whole body cryotherapy (WBC)), is commonly utilised in an attempt to minimise the negative impact of these symptoms on subsequent performance. Although WBC is becoming increasingly popular, there remains a lack of literature comparing CWI and WBC following ecologically valid exercise stresses and research suggests that reported beneficial effects may be attributed to the placebo effect. Furthermore, there is a need to examine the impact of repeated cryotherapy exposure on adaptations to training. Therefore, the aim of this thesis was to compare the effectiveness of CWI, WBC and placebo interventions on acute recovery following endurance and resistance exercise, and to examine the impact of habitual CWI on adaptations to a resistance training programme.

The acute studies demonstrated that cryotherapy was more effective than a placebo for attenuating increases in muscle soreness. Whilst there was no difference in soreness between CWI and WBC following endurance exercise, WBC was superior to CWI following resistance exercise. Neither cryotherapy intervention was more effective than a placebo for recovery of functional markers following endurance or resistance exercise. Importantly, cryotherapy appeared to exacerbate rather than attenuate the inflammatory response following both exercise modes compared to a placebo.

When used as an adjunct to a resistance training programme, CWI did not attenuate soreness over time, and diminished increases in muscle fibre pennation angle compared to a placebo. CWI may have enhanced neural adaptations, although there were no clear functional improvements compared to placebo. Lastly, decrements in markers of bone and collagen turnover were more pronounced in the CWI group.

These findings indicate that cryotherapy is no more effective than a placebo intervention for functional recovery or the attenuation of inflammation following acute endurance or resistance exercise. Furthermore, whilst CWI may enhance neural adaptations to resistance training, repeated exposure may result in maladaptive hypertrophic responses.

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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.

Name:

Signature:

Date:

Peer reviewed publications arising from thesis

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Paice, K., Dimitriou, L., **Wilson, L.**, Hills, F., Sinclair, S., Gondek, M., Kwan, K., Wood, A. & Cockburn, E. (2017). Influence of cold water immersion and cherry juice on recovery following a 42.2km trail run. *Journal of Sports Sciences,* 35(S1), s92.

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Wilson, L., Cockburn, E., Paice, K., Sinclair, S., Faki, T., Hills, F. & Dimitriou, L. (2016). The effects of two different cryotherapy interventions on markers of recovery following a 26.2 mile trail run. 21st Annual Congress of the European College of Sport Science, Vienna. 6th-9th July.

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Table of Abbreviations

5-HPETE	5-Hydroperoxyeicosatetraenoic Acid
5-LO	5-lipoxygenase
Ca^{2+}	Calcium Ion
COX	Cyclooxygenase
CK	Creatine Kinase
CMJ	Counter Movement Jump
CRP	C-Reactive Protein
CWI	Cold Water Immersion
DALDA	Daily Analysis of the Lifestyle Demands of Athletes
DXA	Dual X-ray Absorptiometry
ELISA	Enzyme-Linked Immunosorbent Assay
FLAP	5-LO activated protein
IL	Interleukin
LTA4	Leukotriene A4
LTA4H	LTA4 Hydrolase
LTB4	Leukotriene B4
MAFbx	Muscle Atrophy F-Box
mTOR	Mammalian Target of Rampamycin
MuRF1	Muscle Ring Finger 1
MVIC	Maximal Voluntary Isometric Contraction
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NFκB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
PINP	Procollagen I N-terminal Peptide
PIIINP	Procollagen III N-terminal Peptide
PBC	Partial Body Cryotherapy
PLA	Phospholipases
PL	Placebo
QS	Quantitative Sandwich
RFD	Rate of Force Development
RM	Repetition Maximum
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RSI	Reactive Strength Index
TNF-α	Tumour Necrosis Factor-α
Ub	Ubiquitin
UPP	Ubiquitin Proteasome Pathway
WBC	Whole Body Cryotherapy

Chapter 1

Introduction

1.1. Introduction

It is well documented that exercise involving repeated muscle actions can result in structural damage to muscle fibres and the initiation of an inflammatory cascade (Bernecker et al., 2013; Clarkson & Hubal, 2002; Finsterer, 2012; Stanković & Radovanović, 2012). This is especially true when the exercise stress is novel, or of a greater intensity or duration than that to which the individual is accustomed (Leeder, Gissane, van Someren, Gregson, & Howatson, 2012; Rose, Edwards, Siegler, Graham, & Caillaud, 2017; White & Wells, 2013). The practical implications of these physiological stresses can include temporary reductions in force generating capacity (Abaïdia et al., 2016; Hohenauer et al., 2018; Peñailillo, Blazevich, Numazawa, & Nosaka, 2015), increases in muscle soreness (Cheung, Hume, & Maxwell, 2003; Costello, 2013; Gulick & Kimura, 1996), and stiffness or swelling that can last for a number of days following the exercise bout (Cheung et al., 2003; Jakeman, Byrne, & Eston, 2010; Leeder et al., 2014). All of these symptoms can impact subsequent exercise performance where recovery is insufficient or ineffectively implemented (Hausswirth et al., 2014; Ihsan, Watson, & Abbiss, 2016; Mawhinney & Allan, 2018; Nédélec, Halson, Abaidia, Ahmaidi, & Dupont, 2015).

For those involved in competitive sport, the ability to repeatedly perform at an optimal level, either in training or competitive situations, can delineate success and failure. Therefore, athletes routinely utilise interventions in an attempt to expedite recovery and attenuate the changes observed following strenuous exercise (Bahnert, Norton, & Lock, 2013; Halson, 2013; Ihsan, Watson, & Abbiss, 2016; Nédélec, Halson, Abaidia, Ahmaidi, & Dupont, 2015; Vaile, Halson, & Graham, 2010). Cryotherapy is a popular recovery strategy, and refers to whole body cryotherapy (WBC), partial body cryotherapy (PBC), cold water immersion (CWI), application of crushed ice or ice packs, ice massage or any other mode of cold application for therapeutic purposes (White & Wells, 2013). Cryotherapy, in its

various forms, has long been used to relieve pain and inflammation (Nadler, Weingand, & Kruse, 2004), but is now commonly utilised in a sporting context. Cryotherapy is a popular recovery intervention strategy employed by athletes from a wide variety of sports in an attempt to attenuate the potential negative impact of strenuous exercise on subsequent performance (Bleakley, McDonough, & MacAuley, 2004; Hubbard, Aronson, & Denegar, 2004; Meeusen & Lievens, 1985).

Several different mechanisms have been proposed in an effort to clarify the potential benefits cryotherapy could offer. Leeder and colleagues (2012) stated that the mechanisms of CWI are most likely related to temperature- and pressure-induced changes in blood flow and muscle temperature, leading to an attenuation of post exercise inflammation. More specifically, putative recovery mechanisms may include analgesia, the mitigation of hyperthermia mediated fatigue, decreases in muscle soreness, reductions in the inflammatory response and removal of accumulated metabolic by-products from muscle tissues (Ihsan et al., 2016). The proposed mechanisms relating to WBC are largely similar (Rose et al., 2017), although the element of hydrostatic pressure is eliminated.

Whilst there are numerous cryotherapy modalities, CWI remains a popular choice for many athletes and coaches, possibly due in part to the relative ease of use. CWI is easy to administer and requires no specialist equipment or training; simply a container, bath, or even a large plastic bin filled with ice and water in which to immerse athletes (McGorm, Roberts, Coombes, & Peake, 2015; Wilcock, Cronin, & Hing, 2006). The use of WBC as an alternative to more traditional cryotherapy applications such as CWI is increasing (Hohenauer et al., 2018; Holmes & Willoughby, 2016; Krüger, de Mareés, Dittmar, Sperlich, & Mester, 2015; Lombardi, Ziemann, & Banfi, 2017). Many top level clubs and teams across a variety of sports are now taking advantage of specially built temperature controlled chambers to try and maximise any potential benefits elicited by cold application following exercise

(Bleakley, Bieuzen, Davison, & Costello, 2014). A common assumption is that the extreme nature of WBC offers significant advantages over traditional methods of cooling such as CWI (Bleakley et al., 2014).

To date, there is little scientific evidence supporting the use of WBC as an alternative to CWI. In fact, in terms of functional recovery, there only appears to be one study that has directly compared CWI and WBC (Abaïdia et al., 2016). There is literature investigating the capacity of each modality to cool tissue and the impact that may have on peripheral blood flow (Mawhinney et al., 2017), but the findings are yet to be contextualised in relation to recovery and performance. WBC remains a less accessible and far more expensive treatment than CWI (Poppendieck, Faude, Wegmann, & Meyer, 2013) and therefore it is pertinent to compare the efficacy of each method to allow athletes and practitioners to make informed decisions about the implementation of cryotherapy recovery strategies.

The specificity of recovery interventions is an area receiving an increasing amount of attention (Minett & Costello, 2015; Stephens et al., 2016). Physiological changes can be initiated via different pathways depending upon the type of exercise stress experienced (Armstrong, 1990; Armstrong, Warren, & Warren, 1991; Tee, Bosch, & Lambert, 2007). With this in mind, it follows that recovery intervention selection should be targeted to address the specific mechanisms responsible for disturbances in perceptual responses and functional markers; however, this is not always the case. As such, there is a need to investigate the efficacy of cryotherapy interventions following different exercise stresses. The purported success of cryotherapy interventions is largely based on the understanding that cold application may modulate some of the physiological responses to strenuous exercise in order to facilitate recovery. However, where such interventions are used on a habitual basis, there is a need to understand the potential impact of interrupting the accepted cycle of damage and repair that underlies adaptation to a training stimulus.

Another important aspect that is gaining increasing attention in the literature is the potential impact of placebo effects within sport and exercise science research (Beedie et al., 2018). The placebo effect relates to a desirable outcome resulting from a person's expected or learned response to a treatment (McClung & Collins, 2007). Whilst placebo-controlled study designs are commonplace in clinical research (Beedie et al., 2018; Finniss, Kaptchuk, Miller, & Benedetti, 2010), they are not always adopted within sport science. Whilst there are many discrete neurobiological mechanisms responsible for placebo effects, in the context of sport performance and recovery research, the outcome is often more important (Beedie et al., 2017). Cryotherapy research poses a particular challenge as it is almost impossible to effectively blind participants to their treatment intervention. As a result, the majority of available cryotherapy literature has not implemented a placebo condition, making it difficult to assess the contribution of placebo effects to the success of any cryotherapy application. Therefore there is scope to examine the effectiveness of cryotherapy as a recovery intervention in comparison to a placebo condition.

Given the increasing popularity of cryotherapy, the overall aim of this thesis was to add to the current body of knowledge relating to the effects of acute and habitual cryotherapy exposure on recovery and adaptation following strenuous exercise, using placebo-controlled study designs. Specifically, the individual study chapters aimed to:

1) Establish whether cryotherapy (CWI or WBC) is any more efficacious than a placebo intervention for recovery following an acute bout of strenuous exercise.

2) Determine whether WBC is any more effective than CWI for recovery following an acute bout of strenuous exercise.

3) Establish whether exercise mode influences the effectiveness of cryotherapy application.

4) Investigate whether repeated cryotherapy exposure during an ecologically valid strength and power training programme influences training adaptations compared to a placebo intervention.

Study 1 investigated the influence of CWI, WBC or a placebo intervention on recovery following completion of a 42.2 km trail marathon run. Healthy endurance trained males were recruited for the study and functional and perceptual responses, as well as inflammatory and intramuscular protein markers were assessed immediately before, and up to 48 hours following cessation of exercise.

Study 2 utilised a similar study design but examined the effectiveness of CWI, WBC or a placebo intervention on recovery following a resistance exercise session. Healthy strength trained males were recruited for the study and changes in muscle function, perceptual responses as well as inflammatory and intramuscular protein markers were assessed from baseline up to 72 h post exercise.

Finally, study 3 investigated the influence of repeated CWI exposure or a placebo intervention on adaptations to training. Strength trained participants completed two 4 week blocks of a resistance training programme consisting of high load lower body strength and power training, and completed their allocated recovery intervention after each session. Changes in muscle architecture, muscle function, fibre recruitment, perceptual responses and markers of bone and collagen turnover were assessed at baseline, mid-point and post training.

Chapter 2

Literature Review

2.1. Introduction

This review will discuss literature relating to the complex physiological mechanisms that underpin many of the physiological, perceptual and biochemical markers associated with strenuous exercise. The review will then move on to examine the role of cryotherapy as a recovery intervention, and critically evaluate the current body of literature relating to the use of cryotherapy following strenuous exercise. Whilst the term cryotherapy can be used to describe a number of different modalities, for the purpose of this review, cryotherapy has been categorised as either CWI or WBC.

2.2. Physiological stress from strenuous exercise

Following strenuous exercise, it is common for athletes to experience symptoms of muscle damage, inflammation, oxidative stress and fatigue (Bernecker et al., 2013; Clarkson & Hubal, 2002; Finsterer, 2012; Stanković & Radovanović, 2012). These physiological stresses can manifest as reduced performance potential, likely to result from increased muscle soreness and decreased muscle function (McHugh, Connolly, Eston, & Gleim, 1999), as well as increased stiffness and swelling that can last for a number of days following the initial insult (Armstrong, 1984). Whilst exercise induced muscle damage (EIMD) is a term commonly used to describe symptoms individuals experience after exercise (Byrne, Twist, & Eston, 2004; Clarkson & Sayers, 1999; Clarkson & Hubal, 2002; McHugh et al., 1999; Tee, Bosch, & Lambert, 2007), it does not adequately reflect or encompass the multifaceted physiological and psychological responses. For the purpose of this thesis, where the term ‘muscle damage’ is used, it refers solely to the physiological and structural changes that are evident following strenuous exercise. Muscle damaging exercise can take the form of novel exercise, eccentric muscle actions or exercise of a greater duration or intensity than that to which an individual is

accustomed (Byrne et al., 2004; Cheung, Hume, & Maxwell, 2003; Leeder et al., 2014). Whilst eccentric biased exercise is often used as a model to study physiological responses to strenuous exercise (Byrne & Eston, 2002; Friden & Lieber, 1992; Lieber & Fridén, 1999), damage and inflammation can also occur following concentric contractions or repetitive closed chain movements such as long distance cycling (Bell, Stevenson, Davison, & Howatson, 2016; Hill, Howatson, van Someren, Leeder, & Pedlar, 2014; Ihsan, Watson, & Abbiss, 2016). There is a wealth of literature surrounding the physiological response to unaccustomed or prolonged exercise, but the precise sequence of events remains to be elucidated (Armstrong, 1984; Clarkson & Sayers, 1999; Friden & Lieber, 1992; Lieber & Fridén, 1999; Proske & Morgan, 2001; Pyne, 1993). Armstrong (1990) produced a theoretical model of the physiological responses to strenuous exercise, detailing the complex interrelationship between 4 main stages; initial, autogenic, phagocytic and regenerative. A slightly simplified model was later produced by Kendall and Eston (2002) and is reproduced in figure 2.1 below. For the purposes of this review, the process will be further simplified into a primary and secondary phase. The primary phase is characterised by direct damage to the muscle during the exercise bout (Proske & Morgan, 2001) and the secondary phase refers to the autogenic and phagocytic processes which exacerbate damage caused by the initial insult (Armstrong, 1990). Processes relating to the adaptive and regenerative phase will be discussed in section 2.5.

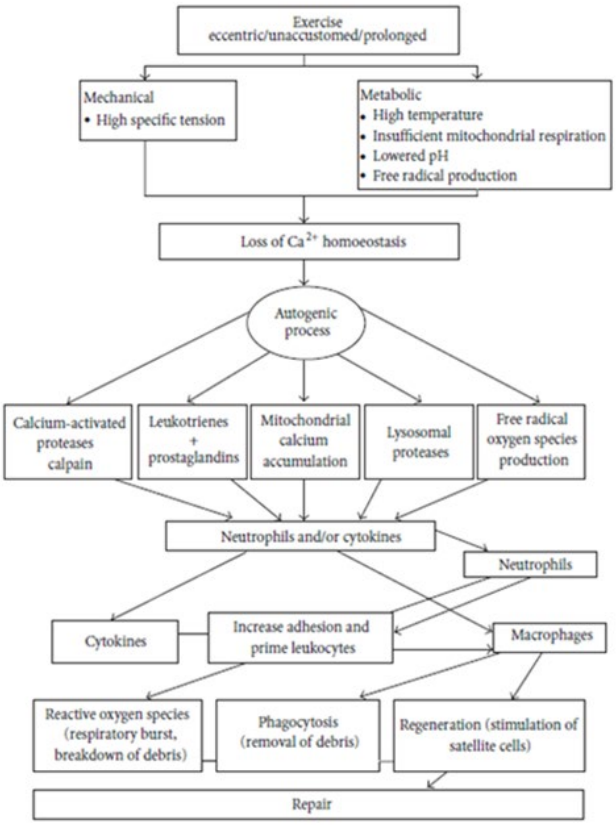

Figure 2.1. Theoretical model of muscle damage and repair cycle reproduced from Kendall & Eston 2002.

2.2.1. Primary event

There are two competing hypotheses pertaining to the primary event and these theories are specific to the type of exercise stress. Broadly speaking, these can be divided into mechanically induced and metabolically induced mechanisms (Armstrong et al., 1991). Whilst researchers have often attempted to investigate the mechanical and metabolic theories in isolation by using extreme exercise protocols, it is likely that both mechanical and metabolic mechanisms may play a role in muscle damage sustained during exercise (Tee et al., 2007). However, the magnitude of damage that can be attributed to either mechanism will largely depend upon the mode of exercise. Similarly, the underlying physiological processes, measured responses and even efficacy of any recovery intervention may differ depending on the initial exercise stress.

2.2.1.1. Mechanical damage theory

The mechanical damage theory refers to damage caused by direct mechanical loading on the muscle fibres during exercise (Byrne et al., 2004; Friden & Lieber, 1992; Proske & Morgan, 2001). This model is usually discussed in terms of eccentric muscle actions and as such, focuses on the unique nature of eccentric, compared to concentric or isometric muscle actions.

Eccentric muscle actions are involved in all locomotor movements such as running and jumping where the muscles exert a braking force to control motion of the body (Whitehead, Allen, Morgan, & Proske, 1998). During eccentric muscle actions, greater tension is produced than during concentric or isometric contractions for a given load (Mchugh, Tyler, Greenberg, & Gleim, 2002). Although muscle tissue is extremely elastic, exercise can result in structural damage within muscle cells (Friden & Lieber, 1992) that can result in functional impairment. Hesselink, Kuipers, Geurten and Van Straaten (1996) demonstrated that reductions in peak isometric torque were more pronounced following a comparable amount of eccentric versus isometric contractions. Further, research shows that eccentric muscle actions preferentially damage fast twitch (type II) muscle fibres, with much greater mechanical disruption compared to slow twitch (type I) muscle fibres (Fridén, Seger, & Ekblom, 1988; Macpherson, Schork, & Faulkner, 1996). Therefore, mechanical muscle damage appears to be the result of high mechanical strain upon a small number of preferentially recruited muscle fibres.

Morgan (1990) proposed that ultrastructural damage of the muscle fibres occurs as a result of non-uniform distribution of sarcomere length change during the eccentric action. This can lead to over-extension of some sarcomeres within the muscle fibres, and eventually cause damage to the fibres themselves (Armstrong, Ogilvie, & Schwane, 1983; Newham, McPhail, Mills, & Edwards, 1983; Ogilvie, Armstrong,

Baird, & Bottoms, 1988). The notion that there is a progressive development of sarcomere inhomogeneities on the descending limb of the length-tension curve has previously been reported (Gordon, Huxtey, & Julian, 1966). Morgan (1990) proposed that during active stretch of a muscle, most of the length change is taken up by the weakest half sarcomeres. On the descending limb of the length tension curve, these sarcomeres become progressively weaker until the fall in active tension is compensated by an increase in passive tension. For some fibres, this may correspond with the point at which there is no longer any myofilament overlap (Proske & Morgan, 2001). Other sarcomeres are then required to take on the stretch to allow further lengthening. This process is repeated with the next weakest sarcomere stretching and so on. This process is often referred to as the popping sarcomere hypothesis (Morgan, 1990; Morgan & Proske, 2004). It is thought that these overstretched sarcomeres are located at random points along the length of the muscle fibre (Balnave, Davey, & Allen, 1997; Proske & Allen, 2005). It is thought that once one or more sarcomeres have become disrupted, damage may spread longitudinally or transversely to adjacent fibres (Proske & Allen, 2005). At the end of the stretch, many of the overstretched sarcomeres are able to resume their normal function whilst some are rendered useless (Talbot & Morgan, 1996). After repeated eccentric muscle actions the number of disrupted sarcomere units increases until the point is reached where the muscle fibre is no longer able to function and membrane damage occurs (Figure 2.2).

Membrane damage itself can be envisaged as a two-stage process, beginning with tearing of t-tubules. This is followed by damage to the sarcoplasmic reticulum, resulting in uncontrolled calcium (Ca^{2+}) release into the intracellular fluid and the initiation of a number of secondary processes (Proske & Morgan, 2001) which will be discussed in section 2.2.2. If damage becomes extensive enough, parts of, or the whole fibre will die. There does not appear to be any evidence to suggest that

the muscle is permanently impaired after mechanical damage; rather it is a normal precursor to muscle adaptation in response to an exercise stimulus (Armstrong, 1984, 1990).

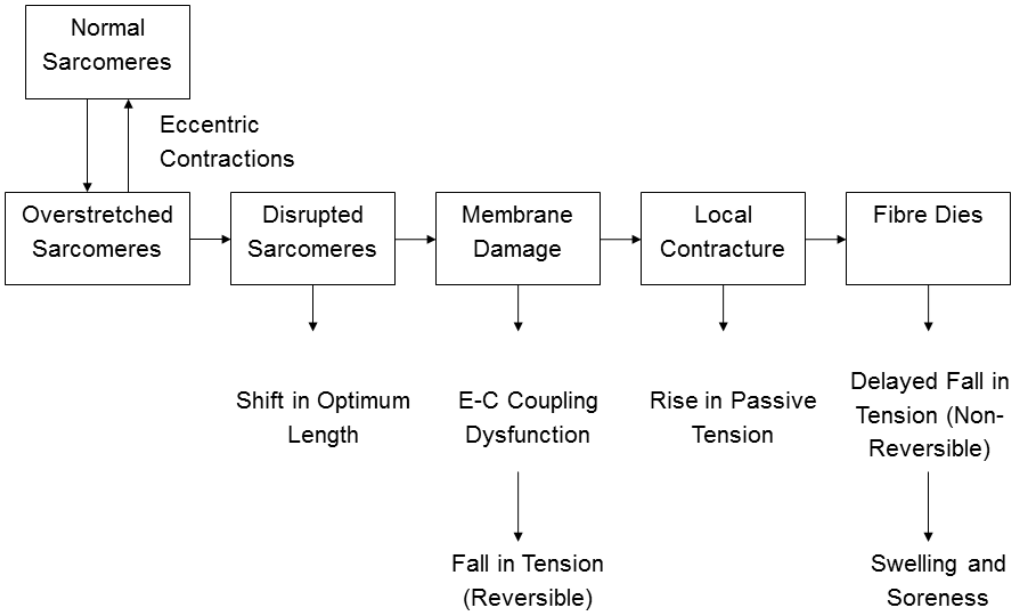

Figure 2.2. Hypothesis for the process of sarcomere disruption following eccentric exercise, taken from Proske and Morgan (2001) p. 334

Evidence of ultrastructural damage has been reported in previous studies (Friden, Sjöström, & Ekblom, 1983; Newham, McPhail, et al., 1983), however it is beyond the scope of this thesis to discuss these findings in detail.

However, an alternative theory was offered by Warren and colleagues (Warren, Ingalls, Lowe, & Armstrong, 2002) after suggesting that histopathological analysis (often considered the 'gold standard' marker of muscle injury) cannot be used to accurately predict magnitude of strength loss. Instead, the authors propose that up to 75% of strength losses apparent following eccentric contractions may be ascribed to a failure of the EC coupling pathway. The first evidence to support this theory came from Warren et al. (1993) who examined force losses in injured muscle isolated from mice. Their findings demonstrated that caffeine-elicited force

production from injured muscles was not different from control muscles despite differences in isometric strength loss (43 and 4% respectively). Given that caffeine acts to increase free cytosolic Ca^{2+} levels in muscle fibres by promoting opening of the SR Ca^{2+} release channel, the authors concluded that force loss occurred as a result of EC coupling failure, rather than damage to force-bearing elements. A further study utilising an in vivo model over a longer time frame (up to 14 days post injury) supported these findings (Ingalls, Warren, Williams, Ward, & Armstrong, 1998). However, Warren et al. (2002) acknowledge that most of the data pertaining to this theory are derived from animal studies utilising electrically stimulated contractions, therefore the applicability to human studies utilising voluntary contractions may be limited.

2.2.1.2. Metabolic damage theory

Tee et al. (2007) suggested that the majority of muscle damage and recovery research has used exercise protocols that aim to isolate eccentric muscle actions, and that the mechanical damage hypothesis cannot account for symptoms observed following exercise that is predominantly concentric in nature, such as long distance cycling. An alternative theory for the primary cause of muscle damage is the metabolic damage theory. The metabolic stress model proposes that the primary event in the process of damage is initiated by metabolic deficiencies in the working muscle, or that such deficiencies render the muscle fibres more susceptible to mechanical damage (Tee et al., 2007). There are several theories encompassed by this model including the production of reactive oxygen- or nitrogen species, insufficient mitochondria respiration and high temperatures (Armstrong et al., 1991; Kendall & Eston, 2002; Moflehi, Kok, Tengku-Fadilah, & Amri, 2012; Viña et al., 2001). Whilst some research suggests that mitochondrial respiration is the most important source of free radical formation during high intensity exercise (Halliwell, 1994, 1998), more recently it has been suggested that a drop in mitochondrial

oxygen partial pressure rather than altered oxygen flux may also cause enhanced formation of superoxide during exercise (Jackson, Pye, & Palomero, 2007). Inadequate coupling of the electron transfer chain can result in a decrease of mitochondrial respiratory control, failure in structural integrity of the sarcoplasmic reticulum and leakage of electrons to oxygen resulting in the formation of superoxide species (Davies, Quintanilha, Brooks, & Packer, 1982; Moflehi et al., 2012; Niess & Simon, 2007; Rattray, Caillaud, Ruell, & Thompson, 2011). Given that metabolic rate can increase by 100 fold during exercise compared to resting values, a considerable increase in oxygen flux through mitochondria has been considered as a major mechanism of exercise-induced formation of reactive oxygen species (ROS), even in highly trained athletes (Moflehi et al., 2012).

ROS or reactive nitrogen species (RNS) are atoms or molecules which have one or more unpaired valence electrons, making them highly chemically reactive (Niess & Simon, 2007). At a cellular level, ROS regulate growth, apoptosis and other cell signalling functions, and at a systems level they contribute to a number of complex functions including blood pressure regulation and cognitive and immune function (Brieger, Schiavone, Miller, & Krause, 2012). Further, ROS are required for crosslinking extracellular matrices and facilitate within- and between cell communications. Maintenance of cellular redox homeostasis requires a balance between the generation rate of ROS/RNS and the capacity of the antioxidant system. Acute physical exertion has been shown to induce an increased generation of ROS/RNS in skeletal muscle (Ji, 1999; Sahlin et al., 2010; Vollaard, Shearman, & Cooper, 2005). ROS/RNS are also implicated in the modulation of contractile function, meaning skeletal muscle is both a generation site and a target in the context of exercise (Niess & Simon, 2007). When ROS/RNS generation is increased beyond the capacity of the antioxidant system during strenuous exercise, cellular redox homeostasis is impaired. Such imbalances can lead to modifications of lipids,

proteins, DNA and other cellular compounds (Halliwell & Cross, 1994; Sjödin, Westing, & Apple, 1990; Tiidus, 1998), leaving cells vulnerable to toxicity and damage. Whilst alterations to DNA structures tend to be implicated in long term clinical pathologies, lipid and protein peroxidation are of particular pertinence in relation to acute response to exercise. As already alluded to, the breakdown of cell structures can result in force loss, a disruption of the excitation-contraction (EC) coupling system and an acute inflammatory response (Bell, McHugh, Stevenson, & Howatson, 2014; Byrne et al., 2004; Tee et al., 2007).

Sahlin et al. (2010) proposes that the process of metabolic damage is a vicious cyclical process whereby oxidatively damaged mitochondria increase their ROS production, leading to further damage and thus increased production of ROS and even further damage. This continual process also leads to exacerbation of secondary damage which will be discussed in the following sections.

2.2.2. Secondary event

As already alluded to, whether the primary event is initiated by mechanical or metabolic factors, a secondary stage which exacerbates the effects of damage is evident. The most commonly accepted mechanism is that of a disturbance in Ca^{2+} homeostasis and resultant rise in cytosolic Ca^{2+} which can trigger the activation of a number of degradative pathways (Tee et al., 2007). These mechanisms include the activation of Ca^{2+} dependent proteolytic and phospholipolytic pathways which can degrade structural and contractile myofibre proteins as well as the myofibre membrane (please refer to figure 2.2). These processes will be discussed in the following sections.

2.2.2.1. Disturbance of Ca^{2+} homeostasis

In order for skeletal muscles to contract, action potentials generated at the neuromuscular junction travel along the surface membrane of muscle fibres and

trigger a transient release of Ca^{2+} from the sarcoplasmic reticulum (SR). The brief rise allows for the formation of crossbridges and results in fibre contraction. Reuptake of free Ca^{2+} into the SR restores intracellular Ca^{2+} to resting levels and the muscle relaxes (Place, Yamada, Bruton, & Westerblad, 2010).

Once levels of free cytosolic Ca^{2+} exceed the working capacity of buffering systems within the cell or are elevated for prolonged periods of time (longer than the transient rises seen during normal fibre contraction), a number of Ca^{2+} activated degradative mechanisms are initiated within the muscle fibres (Armstrong et al., 1991).

Under normal circumstances (i.e. in the absence of cell membrane damage or muscle fibre injury), cytosolic levels of Ca^{2+} are closely regulated by a number of buffering and translocation mechanisms present in the cell (Tee et al., 2007; Tibbits & Thomas, 1989). Although Ca^{2+} increases during muscle contraction, such increases do not normally elicit structural damage. There are a number of mechanisms that may explain this phenomenon. Firstly, free Ca^{2+} is rapidly bound to troponin or other calcium binding proteins within the cell, or is removed by transmembrane ATPase pumps (Patergnani et al., 2011) meaning changes in Ca^{2+} concentration are transient (Robertson, Johnson, & Potter, 1981). Secondly, it has been suggested that degradative enzymes are compartmentalised within the cell meaning that there is no interaction with Ca^{2+} involved in EC coupling (Armstrong et al., 1991). However, concentrations of extracellular Ca^{2+} are far greater than cytosolic concentrations (2-3 mmol/L compared to 0.1 µmol/L) so any change in membrane permeability resulting from sarcolemmal disruption or tearing (evidenced following exercise) would allow for a huge influx of Ca^{2+} from the interstitial fluid into the cell (Armstrong et al., 1991). At this point, the buffering and translocation mechanisms within the cell may become overwhelmed leading to an accumulation of free Ca^{2+} within the cell. A significant and sustained increase in intracellular Ca^{2+}

would then activate a number of degradative pathways. Duncan (1987) proposed that whilst absolute Ca^{2+} level is an important moderator of cell degradation, ultimately the duration and magnitude of active movement across key membranes within the fibre is the critical determining factor.

A number of studies have demonstrated increased intracellular Ca^{2+} levels following exercise (Azenabor & Hoffman-Goetz, 2000; Balnave & Allen, 1995; Byrd, 1992; Duan, Delp, Hayes, Delp, & Armstrong, 1990; Kuipers, 1994; Mooren, Lechtermann, Fromme, Thorwesten, & Völker, 2001) suggesting that Ca^{2+} disturbance is implicated in the process of muscle fibre damage. Whilst a number of studies used rat or mouse models, Mooren et al. (2001) demonstrated increased levels of intracellular Ca^{2+} immediately following a high intensity (80% $\dot{V}O_2$ max) treadmill run to exhaustion in human participants. Further evidence supporting the role of intracellular Ca^{2+} as a moderator of skeletal fibre damage comes from Soza, Karpati, Carpenter, & Prescott (1986) and Beaton, Tarnopolsky, & Phillips (2002) who studied the effect of calcium channel blockers on muscle damage in rat and human fibres respectively. Soza et al. (1986) reported that there was a distinct rise in CK levels in the control sample, but no rise in CK activity in those samples that had been treated with a calcium blocking solution. Similarly, Beaton et al. (2002) reported that those participants who had been consuming calcium channel blockers for 7 days pre- and post- exercise, demonstrated far lower levels of Z-band streaming 4 h after completion of an eccentric muscle damaging exercise than those in the control group.

There are a number of mechanisms that have been proposed that may account for observed increases of intracellular Ca^{2+} following exercise. First, any alterations to the structural integrity of cell membranes as a result of mechanical damage can lead to changes in membrane permeability (Paulsen, Mikkelsen, Raastad, & Peake, 2012). This may result in an efflux of Ca^{2+} across the membrane into the

intracellular space due to the concentration gradient differential (Armstrong et al., 1991). This rapid increase of Ca^{2+} would likely overwhelm the normal buffering systems within the fibre (Armstrong et al., 1991). A further possible contributor to increased cell membrane permeability following muscle fibre injury is the involvement of stretch-activated channels (Yeung & Allen, 2004). Stretch-activated ion channels are stimulated and opened in response to mechanical stress in the cell membrane, resulting in an increased ionic flow (see figure 2.3). Mechanosensitive channels can be selectively (passing only K^+ or Cl^-) or non-selectively (passing Ca^{2+}, Na^+ and K^+) permeable. Given that the stretch-activated channels present in the sarcolemma have been shown to be non-specific (non-selectively permeable) cation channels allowing influx of Ca^{2+}, this pathway could contribute to increased Ca^{2+} levels following fibre injury (Yeung & Allen, 2004).

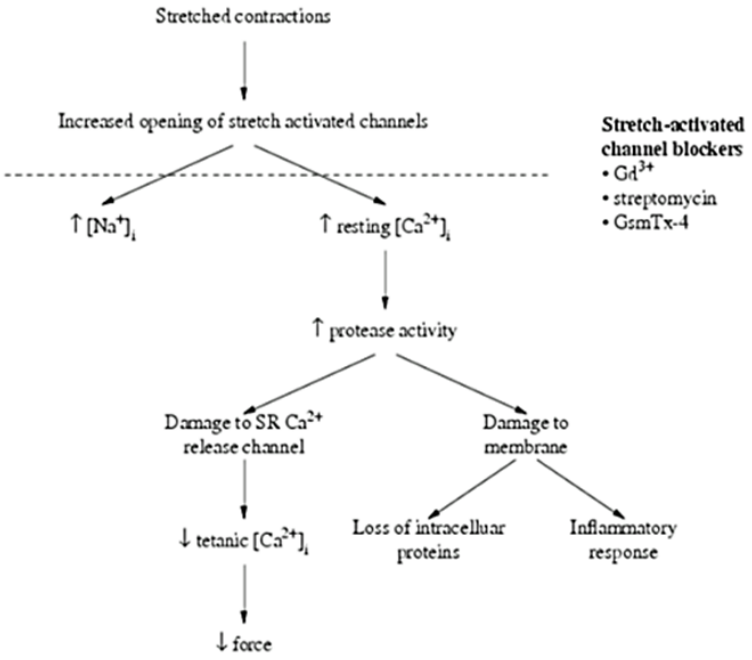

Figure 2.3. Working hypothesis of stretch-activated channels, ionic changes and muscle damage. Reproduced from Yeung & Allen (2004).

Similarly, the depletion of ATP through sustained exercise can result in increased intracellular Ca^{2+} due to dysfunction or failure of Ca^{2+} ATPase pumps (Baird,

Graham, Baker, & Bickerstaff, 2012). Byrd and colleagues (Byrd, Bode, & Klug, 1989; Byrd, McCutcheon, Hodgson, & Gollnick, 1989) demonstrated that exhaustive moderate and high intensity exercise reduced the ability of the SR to sequester Ca^{2+} in both rat and horse muscle fibres.

The disturbance of Ca^{2+} homeostasis is considered the next sequential step in the process of muscle fibre injury following the primary event (Armstrong et al., 1991). The following sections will discuss some of the pathways that may be activated when Ca^{2+} homeostasis is disturbed.

2.2.2.2. Calpain

Calpain is an intracellular Ca^{2+} dependant proteolytic enzyme found at the Z-lines and M-bands of muscle cells (Azmy & Abdallah, 2013). Calpain is responsible for the breakdown of protein substrates, including cytoskeletal and myofibrillar proteins (Belcastro, Shewchuk, & Raj, 1998). An increase in cytosolic Ca^{2+} initiates calpain activation and an upregulation in the rate of calpain-mediated degradation within the cell, resulting in impairment of mitochondrial function (Kar et al., 2010; Rattray, Thompson, Ruell, & Caillaud, 2013). Whilst numerous calpains and calpain-like molecules have been identified in a variety of organisms (Goll, Goll, Thompson, Li, & Wei, 2003), in terms of skeletal muscle, there are 3 isoforms of interest; calpain-1 (μ-calpain), calpain-2 (m-calpain), and calpain-3 (expressed exclusively in skeletal muscle) (Gissel, 2005).

Ultra-structural damage in the form of Z-line streaming is commonly reported following strenuous exercise (Friden et al., 1983; Newham, McPhail, et al., 1983; Proske & Morgan, 2001). There is evidence to suggest that calpain is implicated in this process (Cunha et al., 2012; Ma et al., 2011; Zhang et al., 2012) although this evidence comes from animal studies, possibly due to challenges associated with in vivo sampling in humans (Kerksick, Willoughby, Kouretas, & Tsatsakis, 2013).

However, the upregulation of calpain activity following exercise has also been evidenced in human studies. Murphy et al. (2007) and Raastad et al. (2010) examined muscle biopsies taken from the vastus lateralis of human participants before and after completion of unilateral maximal voluntary eccentric actions of the knee extensors. Murphy et al. (2007) reported that although unchanged immediately and 3 hours post, autolysed calpain-3 was markedly increased (from ~16% to ~35% of total calpain-3) 24 hours following exercise. Conversely, Raastad et al. (2010) demonstrated that calpain activity was three times higher in the exercised limb compared to the control limb 30 min after exercise and remained significantly higher up to 96 hours post exercise. The differences seen in the results of these 2 studies may be explained by differences in the method of analysis used to detect the presence of calpain (colourimetric assay versus western blot).

As well as being directly implicated in the process of protein breakdown, it is likely that calpain also interacts with a number of pro-inflammatory cytokines to elicit further degradation via cytokine pathways. A study by Lokuta, Nuzzi, & Huttenlocher (2003) examined the role of calpain in neutrophil chemotaxis. By establishing a chemoattractant gradient using interleukin (IL) -8, the authors investigated whether calpain inhibition attenuated neutrophil migration. Their results showed that chemotactic migration was reduced following calpain inhibition, with the authors suggesting that calpain is required to achieve optimal chemotactic migration towards IL-8. Further, Belcastro et al. (1998) suggested that whilst calpain-mediated degradation of muscle tissue following exercise is fairly well evidenced (Zhang et al., 2012), there is scope to further investigate the involvement of calpain in the inflammatory and ultimately regenerative responses to exercise.

2.2.2.3. Cytokines

Cytokines are small secreted proteins released by cells that have a specific effect on interactions and communications between cells. The acute-phase response to strenuous exercise is characterised by the secretion of cytokines from local inflammatory cells (neutrophil granulocytes and macrophages) into the bloodstream. ILs are cytokines made by one leucocyte which act on other leucocytes (Zhang & An, 2007). Some of the most commonly reported acute-phase markers following strenuous exercise are IL-6, C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α) (Zhang & An, 2007). The specific responses of these inflammatory mediators to different exercise modes will be discussed in detail in section 2.3.6.

An upregulation of cytokines initiates the proliferation and infiltration of neutrophils and macrophages to the damaged area (Pyne, 1994). This in turn may initiate or exacerbate a number of degradative pathways, therefore IL-6, TNF-α, and CRP are implicated in the mediation of the phagocytic phase of the damage/regeneration process. The generation and activation of ROS may also contribute to the secondary phase of damage and this will be discussed in further detail below.

2.2.2.4. ROS

ROS are implicated in the initial process of muscle damage as part of the metabolic damage theory. However, mitochondrial ROS production can also be activated by an increase of intracellular Ca^{2+} or by the production of cytokines (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014). Additionally, the activation of professional phagocytes such as neutrophils is associated with an increase in cellular oxygen consumption (Dahlgren, Karlsson, & Bylund, 2007) and a resultant respiratory or oxidative burst, leading to an increased production of ROS. The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, which is dormant under normal conditions, is activated during respiratory burst. NADPH-oxidase then gives rise to the generation

of superoxide anion and hydrogen peroxide (Dahlgren et al., 2007). Whilst at low concentrations (exact concentrations remain to be defined) ROS provide complex signalling functions, high concentrations can result in protein and lipid oxidation (Mittal et al., 2014). As such, ROS production following disturbances in Ca^{2+} homeostasis can further exacerbate damage caused during the primary phase.

2.2.2.5. Phospholipases

The activation of phospholipases (PLA) and specifically PLA_2 is thought to be initiated by increased intracellular Ca^{2+} concentrations (Belcastro et al., 1998; Cheung, Hume, & Maxwell, 2003; Gissel, 2005). PLA_2 is an enzyme that cleaves mitochondrial and other membrane phospholipids, disrupting membrane lipid organisation and releasing arachidonic acid, which is a substrate for further ROS-generating enzyme systems (Gissel, 2005; Powers, Nelson, & Hudson, 2011). In addition, the activation of PLA_2 can cause further damage to the sarcolemma by increasing production of leukotrienes and prostaglandins which increase protein degeneration at the weakened Z-lines (Cheung et al., 2003). PLA_2 is the first enzyme in the pathway leading to the production of a number of metabolites including prostaglandins and leukotrienes (Armstrong et al., 1991; Bondesen et al., 2004) that are implicated in acute inflammation following muscular contractile activity (Gong et al., 2006; Powers et al., 2011), and can lead to further deleterious effects on cell membrane integrity (Gissel, 2005; McKune et al., 2012).

2.2.2.6. Prostaglandins

Prostaglandins are produced by PLA_2 through the sequential oxidation of arachidonic acid. Prostaglandins are implicated in the process of inflammation and are generated from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes (Ricciotti & FitzGerald, 2011). There are two cyclooxygenase isoforms, COX-1 and COX-2, although COX-2 appears to be the dominant source of

prostaglandin production, both forms appear to be implicated in the acute inflammatory response in humans (Ricciotti & FitzGerald, 2011). It has been suggested that COX-1 is responsible for baseline levels of prostaglandins whilst COX-2 produces prostaglandins through stimulation. Research suggests that COX-1 drives the acute phase of an inflammatory response, with an upregulation of COX-2 occurring within a few hours (Smyth, Grosser, Wang, Yu, & FitzGerald, 2009).

Carroll et al. (2013) investigated COX-1 and COX-2 activity following an acute bout of resistance exercise. Their results showed that COX-1 activity was increased at 4 h post exercise but returned to baseline levels by 24 h post, whereas COX-2 activity remained elevated at 4 and 24 h following cessation of exercise. Similarly, Buford, Cooke, Shelmadine, et al. (2009) reported that 45 min of downhill running at 60% $\dot{V}O_2$ max resulted in a significant upregulation of COX-2 mRNA 3 h post exercise. Much like TNF-α, the formation of prostaglandins via COX-2 appears to have a dichotomous role in terms of muscle degradation and regeneration following strenuous exercise (Bondesen et al., 2004; Burd et al., 2010).

2.2.2.7. Leukotrienes

Leukotrienes are another metabolite of arachidonic acid and are produced via the lipoxygenase pathway (mediated by the arachidonate 5-lipoxygenase (5-LO) enzyme). Arachidonic acid is converted by 5-LO and 5-LO activated protein (FLAP) into 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and leukotriene A4 (LTA4). LTA4 is further metabolised by LTA4 hydrolase (LTA4H) into leukotriene B4 (LTB4) (Korotkova & Lundberg, 2014). Data relating to the role of leukotrienes in humans following strenuous exercise is relatively scarce, however, LTB4 has been detected in healthy human skeletal muscle (Hedenberg-Magnusson, Ernberg, Alstergren, & Kopp, 2001). LTB4 is a powerful chemoattractant which mediates inflammation by recruiting leucocytes to damaged tissue and contributes to muscle regeneration by

accelerating the proliferation and differentiation of myoblasts (Korotkova & Lundberg, 2014).

2.2.2.8. Ubiquitin proteasome pathway

All intracellular proteins are continually being hydrolysed to their constituent amino acids and being replaced by new synthesis (Lecker, 2006). This process serves several homeostatic functions and during normal function, overall protein synthesis and degradation is precisely balanced. In all tissues the majority of intracellular protein degradation occurs via the Ubiquitin (Ub)-proteasome pathway (UPP) (Lecker, 2006). The UPP functions by marking proteins for degradation by 'tagging' them with a polyubiquitin chain. This tagging process leads to their recognition and subsequent cleaving by the 26S proteasome, a multicatalytic protease complex that degrades ubiquitinated proteins to small peptides (Baumeister, Walz, Zuhl, & Seemuller, 1998; Jamart et al., 2012). It is likely that cytokines, and TNF-α in particular, play an important role in the activation and upregulation of the UPP (Buford, Cooke, Shelmadine, et al., 2009).

TNF-α has been shown to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) in muscle cells resulting in an increase of UPP activity (Liao, Zhou, Ji, & Zhang, 2010). NFκB is a transcription factor that mediates the transcription of a number of proteins implicated in the inflammatory response and is thought to increase UPP activity (Hasselgren, 2007; Karin & Ben-Neriah, 2000; Li, Chen, Li, & Reid, 2003). NFκB can also be upregulated as a result of increased intracellular Ca^{2+} and elevated ROS production and accumulation (Liao et al., 2010). Although muscle protein degradation can occur in the absence of ubiquitination, at least three Ub-related pathways are stimulated by activated TNF-α NFκB signalling: the upregulation of Ub- conjugating enzyme E2 and Ub-protein ligating enzymes E3,

the conjugation of Ub and the target protein, and the degradation of ubiquitinated protein by the 26S proteasome (Liao et al., 2010).

The specificity of substrate tagging in the UPP is determined by ubiquitin ligases (Jamart et al., 2012). Both muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) are muscle-specific ligases that have been shown to increase during muscle catabolism (Bodine et al., 2001). Kim et al. (2011) demonstrated a 233% increase of TNF-α 3 hours after completion of a 200 km ultra-endurance race, which was accompanied by a 583% increase in MuRF1 and a 249% increase in MAFbx. A further study by Jamart et al. (2012) reported a 71% increase in MuRF1 following a 24 hour endurance run, but no change in MAFbx. The differences in reported magnitude of change may be explained by the total exercise volume, as on average, participants in the first study ran approximately 25% further than those in the second study (200 vs 149.8 km respectively). Increased MuRF1 has also been demonstrated following resistance exercise with Borgenvik, Apro, & Blomstrand (2012) reporting 20-40% increases 3 hours following 100 repetitions of a leg pressing exercise.

2.2.3. Summary

In summary, long duration or strenuous exercise, irrespective of mode, can lead to a disturbance of Ca^{2+} homeostasis which initiates a number of degradative pathways associated with cellular damage. An increase in intracellular Ca^{2+} can lead to the activation of several calpain mediated processes which result in a breakdown of myofibre proteins and an impairment in mitochondrial function. Calpain also interacts with a number of pro-inflammatory cytokines, to increase cell proliferation to areas of damage. The release of IL-6 and CRP appears to be dependent upon exercise duration, with marked differences between the magnitude of response following endurance and resistance based exercise bouts. Cytokines stimulate the

production of ROS which can further exacerbate structural and functional damage within muscle cells. TNF-α has also been shown to activate NFκB in muscle cells resulting in an increase of UPP activity, and a concomitant increase in protein breakdown. These physiological processes can impact upon a number of functional, perceptual and blood borne markers which will be discussed in more detail in the following sections.

2.3. Markers of recovery following strenuous exercise

Numerous studies have been published in an attempt to describe markers of muscle-damaging exercise and to establish the underlying physiological mechanisms responsible for this phenomenon. Despite ongoing debate, there are numerous measures or 'markers' that can be used in an effort to quantify the symptoms of strenuous exercise. The current body of literature demonstrates that a wide variety of criteria are employed to ascertain the presence or extent of exercise stress and its potential to influence subsequent performance, but all markers can be categorised as either direct or indirect.

Muscle biopsies, assessed with histological analysis (either light, or electron microscopy), are direct measures of structural muscle damage. However, given that biopsy techniques are invasive, and that a small tissue sample may not accurately represent the non-uniform alterations across a whole muscle or fibre (Peñailillo et al., 2015), indirect markers are used more routinely (Clarkson & Hubal, 2002). There are more indirect markers reported in the literature than can be adequately reviewed in detail here, therefore the following sections will focus on those markers which are relevant to the present thesis, and should not be considered an exhaustive list.

2.3.1. Muscle soreness

It is likely that the most commonly reported symptom following strenuous exercise is delayed onset muscle soreness (DOMS) (Cheung et al., 2003). Increases in muscle

soreness have been reported following endurance (Crystal, Townson, Cook, & LaRoche, 2013; Hill et al., 2014; Howatson et al., 2010; Shanely et al., 2013), resistance (Fulford, Eston, Rowlands, & Davies, 2015; Glasgow, Ferris, & Bleakley, 2014; Roberts, Nosaka, et al., 2015; Vaile et al., 2008) and prolonged high intensity intermittent exercise (Bell et al., 2016; Chatzinikolaou et al., 2014; Leeder et al., 2014; Leeder et al., 2015; Singh, Guelfi, Landers, Dawson, & Bishop, 2011; White et al., 2014).

Increases in soreness have been demonstrated following prolonged endurance exercise, and marathon performance specifically. Clifford et al. (2017) and Howatson et al. (2010) reported that in groups of trained runners, soreness peaked immediately post marathon, with a fold increase of ≈ 115 reported by Howatson et al. (2010). Values remained elevated at 24 h in both studies (≈ 46 and ≈ 82-fold respectively) but had returned to baseline by 48 h in the Clifford et al. (2017) study. However, in a study from Hill, Howatson, van Someren, Walshe, & Pedlar (2014) muscle soreness peaked 24 h following marathon completion, with a mean fold increase of ≈ 1.4 in the control group. The participant groups in all three studies were closely matched in terms of age and number of previous marathon completions, and environmental conditions were not largely different between studies. However, average finish times in the Hill et al. (2014) study were much quicker than in the Clifford et al. (2017) and Howatson et al. (2010) studies (03:39:27, 04:28:29 and 04:15:48 hh:mm:ss respectively) which may indicate a greater level of fitness or enhanced training status, which could in turn explain the smaller magnitude of soreness response in the Hill et al. (2014) investigation.

Increased levels of perceived soreness have also been evidenced following high intensity resistance exercise. Pointon, Duffield, Cannon and Marino (2011) and Deschenes et al. (2000) respectively, utilised 6 x 25, and 4 x 25 maximal concentric/eccentric muscle contractions of the knee extensors to investigate the

influence of resistance exercise on muscle soreness in resistance trained males. The results from Pointon et al. (2011) showed that soreness was significantly increased post exercise (≈ 7-fold) and did not return to baseline levels by 48 h, where scores were still approximately 3-fold higher than baseline. Similarly, Deschenes et al. (2000) reported significantly elevated soreness scores for 3 days following exercise, with peak values recorded at 2 days post (≈ 4.5-fold increase) in untrained individuals.

These findings demonstrate that strenuous exercise, irrespective of mode, results in increased soreness responses. However, the time course of recovery appears to be modulated by exercise mode, with soreness persisting for longer following lower limb resistance exercise, compared to prolonged endurance exercise, even in trained individuals.

Many studies use the terms muscle damage and muscle soreness interchangeably but functional impairment is not always accurately reflected in the magnitude of soreness, and the time course of recovery of these distinct elements can also differ (Nosaka, Newton, & Sacco, 2002). DOMS usually occurs as a result of strenuous exercise and symptoms can range from mild discomfort and stiffness on palpation and movement to severe pain that can restrict movement (Cheung et al., 2003). Sensory neurons are triggered by potentially harmful stimuli and send appropriate signals to the brain via the spinal cord. Specific sensory neurons called nociceptors are most commonly associated with the perception of pain, and mechanical nociceptors are stimulated by mechanical deformation or increases in pressure (Scholz & Woolf, 2002). When structural muscle damage occurs at the cellular level, intracellular fluid leaks out into the interstitial space, stimulating the nociceptors and resulting in an increased perception of pain. DOMS usually manifests as localised pain concentrated in the distal portion of the muscle (Armstrong, 1984); the localisation of pain can be attributed to a high concentration of muscle pain

receptors in the connective tissue of the myotendinous region (Newham, Mills, Quigley, & Edwards, 1983).

There is ongoing debate about the mechanism responsible for DOMS and there are a number of opposing theories. One unifying element reported in the literature is that DOMS becomes apparent following structural damage within the muscle fibre or connective tissue (Jones, Newham, & Clarkson, 1987; Jones, Newham, & Torgan, 1989; McKune et al., 2012; Proske & Allen, 2005). A 2003 review by Cheung et al., stated that there are up to 6 theories that have been proposed in order to explain the mechanism of DOMS, namely: lactic acid, muscle spasm, connective tissue damage, structural muscle damage, inflammation and the enzyme efflux theories. The lactic acid and muscle spasm theories have now been largely discredited, and more recent literature has emphasised the potential influence of a variety of noxious chemicals or inflammatory agents on soreness following strenuous exercise (Hedayatpour & Falla, 2012; McKune et al., 2012; Niess & Simon, 2007; Proske & Allen, 2005). Whilst it is beyond the scope of this review to discuss and examine each of the theories in detail, it is most likely caused by a combination of 2 or more of the proposed theories (Cheung et al., 2003), and this in turn will likely be dictated by the mode of exercise.

Subjective measures of perceived muscle soreness can be recorded using questionnaires, numerical scales and visual analogue scales (VAS) (Clarkson, Nosaka, & Braun, 1992; Howell, Chleboun, & Conatser, 1993). A further method of assessing soreness is through the use of palpation or myometers, whereby pain ratings are recorded in relation to an applied force. In order to quantify soreness, unidimensional scales, where individuals rate pain on one evaluative element, such as the VAS or Likert scale, are commonly used to rate DOMS. However, pain has been shown to be multidimensional and subject to influence from sensory, evaluative and affective elements. A paper by Cleather and Guthrie (2007)

compared the use of multidimensional and unidimensional scales in the assessment of DOMS and showed that there was no significant difference between the two different scales. This suggests that unidimensional scales such as VAS and Likert scales are adequate methods of assessing pain following strenuous exercise.

Whilst soreness is a commonly reported symptom of strenuous exercise, it appears to correlate relatively poorly with functional capacity (Byrne et al., 2004). Therefore, soreness data is normally used in conjunction with other perceptual and functional parameters to monitor recovery following strenuous exercise.

2.3.2. Perceptual markers of recovery

In addition to muscle soreness, other subjective perceptual data can provide useful information with regards to the efficacy of specific interventions. Questionnaires are routinely used within the literature as a tool to gather data relating to markers such as mood, stress, fatigue and nutritional status, which are all important elements of holistic recovery.

Although perceptual data is subjective in nature, many commonly used questionnaires also have scoring keys so it is possible to compare data across different groups or even studies. There are countless different questionnaires used to measure a plethora of parameters and as such, it would not be possible to include them all here in sufficient detail as it is beyond the scope of this thesis. The Profile Of Mood States (POMS) questionnaire is widely used, although it is not specifically tailored to athletes. The use of more sport specific questionnaires such as the Recovery-Stress Questionnaire (REST-Q), Total Recovery Scale (TQR) and the Daily Analysis of the Lifestyle Demands of Athletes (DALDA) is becoming more evident both in practice and in the literature (Halson, 2014).

As already mentioned, one example of a sport-specific questionnaire is the DALDA (Rushall, 1990), which can be used to monitor changes in stress and recovery

states in athletes (Coutts, Slattery, & Wallace, 2007). The DALDA is a 2 part questionnaire that aims to identify both sources and symptoms of stress. Part A (9 questions) identifies potential sources of stress (including home life, work, sleep and sports training), whilst part B (25 questions) allows individuals to rate symptoms of stress and fatigue as worse than normal, normal, or better than normal. The DALDA offers an opportunity to explore perceptual recovery using a broader and arguably more holistic approach than simply relying on soreness, which, as already discussed, offers little insight into overall perceptual recovery. Although some studies have successfully utilised the DALDA to monitor fatigue and recovery after exercise, given the relative ease of use and interpretation, there is scope for future research to implement this type of assessment in addition to soreness ratings.

Coutts et al. (2007) investigated a number of different tests used to monitor performance, fatigue and recovery in an effort to provide information about overreaching. The study focused on triathletes who were assigned to either a 'normal training' (NT) or 'intensified training' (IT) group during a 4 week training period and 2 week taper period. The authors reported that there were no differences between time points or groups for scores on part A of the questionnaire. However, in part B, athletes in the IT group reported a significantly greater number of 'worse than normal' responses than the NT group in the final week of the overload training period. These results coincided with decreased maximal 3 km run time trial and 5 bound test performance in the NT group, suggesting that the athletes were functionally over reached. The authors concluded that the DALDA is an effective and practical method for monitoring recovery in athletes, and their findings are in agreement with those reported by Halson et al. (2002) who investigated performance changes and markers of fatigue in cyclists during intensified training. The results of the study demonstrated that DALDA scores were significantly

increased (part ‘B’) in conjunction with a significant decline in maximal power output and a significant increase in time to complete a simulated time trial.

Similar results were reported in team sport athletes by Nicholls, Backhouse, Polman, & McKenna (2009) who investigated stressors and affective states among 16 professional rugby union players over a 4 week period. Their results demonstrated that players reported worse than normal scores for muscular pain, unexplained aches and general weakness the day after a game compared to match day or the day before. Training effort was significantly better than normal on match days compared to either the day before or the day after. The authors suggested that as a result of their findings practitioners and coaches should be encouraged to monitor psychological states throughout the season in order to try and identify early symptoms of overreaching or overtraining before it develops into overtraining syndrome. Coutts, Slattery and Wallace (2007) also propose that the use of questionnaire based monitoring methods is preferable to maximal or submaximal physiological testing which can be fatiguing and impractical for athletes. However, at present, the DALDA questionnaire appears to be overlooked as a tool to monitor acute recovery in the days following an exercise bout. As discussed, the questionnaire is sensitive enough to detect day-to-day changes, and can offer insight into a number of physiological and perceptual symptoms that may otherwise be difficult to measure or quantify.

2.3.3. Muscle function

In terms of sporting performance, the potential deleterious effects of strenuous exercise on subsequent muscle function are of particular interest. Impairment of muscle function, in terms of decreases in strength and power can be an immediate and prolonged effect of strenuous exercise. As with many other symptoms, the magnitude of change is affected by the mode, duration and intensity of exercise.

With that said, reductions in isometric strength appear to be more pronounced than changes in isokinetic strength following strenuous exercise.

Hausswirth et al. (2011) and Peñailillo, Blazevich, Numazawa, & Nosaka (2015) both reported decreases in maximal voluntary isometric contraction (MVIC) following endurance exercise. Their results demonstrated a 9.6 and ~12% decrease following a 48 min simulated trail run and 30 min cycling protocol respectively. Howatson et al. (2010) reported a peak decrement in MVIC of ≈ -28% immediately after completion of a marathon in well trained runners. MVIC remained suppressed at both 24 (≈ -18%) and 48 h (≈ -9%) post exercise.

Similar decrements in isokinetic torque, and larger decrements in isometric torque measurements have been reported following resistance or eccentric biased exercise. Cockburn, Robson-Ansley, Hayes, & Stevenson (2012) reported a ~19% reduction in peak isokinetic torque 48 and 72 h following completion of 6 x 10 unilateral eccentric-concentric knee flexions. These results are supported by Fulford et al. (2015) who reported a 22% decrease in isokinetic peak torque 24 h following 10 x 10 back squats performed at 70% body mass, although there were no follow up measures taken beyond 24 h. Reductions in isometric torque of 30-40% have also been reported following eccentric exercise protocols. Ferreira-Junior et al. (2015), Abaïdia et al. (2016) and Costello, Algar, and Donnelly (2012) reported peak isometric torque decreases of 30, 35 and 40% respectively following lower body exercise protocols consisting of drop jumps or maximal eccentric contractions of the quadriceps or hamstrings. In all three studies, MVIC had not recovered to baseline levels by 72 h post exercise.

These findings demonstrate that greater magnitudes of strength loss (both isometric and isokinetic) are evident following resistance exercise compared to prolonged endurance exercise such as marathon running. Furthermore, these decrements

appear to persist for longer following resistance exercise, often taking more than 72 h to recover to baseline levels.

The remaining variation seen in the results could be attributed to methodological differences. In terms of isokinetic measurement, strength losses may well be movement velocity dependent due to the force-velocity relationship. It is well documented that eccentric muscle actions preferentially damage type II fast twitch muscle fibres (Fridén, Seger, & Ekblom, 1988; Friden et al., 1983). Therefore, it might be assumed that a greater percentage strength loss would be seen at higher angular velocities of movement where type II fibres are predominantly recruited. Research by Friden et al. (1983), Golden and Dudley (1992) and Eston, Finney, Baker and Baltzopoulos (1996) supports these findings and suggests that longer recovery periods are seen at higher angular velocities of movement. Conversely, several studies have reported that strength losses are greater at slower versus faster angular velocities (Deschenes et al., 2000; Michaut, Pousson, Babault, & Van Hoecke, 2002; Twist, Gleeson, & Eston, 2008). One explanation may be reduced voluntary activation via the mechanism of 'central inhibition', where once muscles are damaged and become painful, a reduced voluntary drive prevents even further damage (Gandevia, Allen, Butler, & Taylor, 1996; Ihsan et al., 2016; Michaut et al., 2002). This is despite research which implies that individuals can fully activate painful muscles during maximal voluntary contractions (Gandevia, Herbert, & Leeper, 1998). Finally, several researchers, such as Byrne, Eston, and Edwards (2001) and Twist and Eston (2007), have reported no difference in strength loss between slow and fast velocities.

Reductions in isometric strength following eccentric exercise are also dependent upon the muscle length at which they are measured (Byrne et al., 2001). A reduction in sarcomere length would account for the greatest loss of strength at short muscle lengths. Indeed, greater strength loss at short versus optimal or long

muscle length indirectly supports the hypothesis that the length–tension curve shifts to the right, towards longer muscle lengths following eccentric exercise (Byrne et al., 2004). Measurement angles for peak isometric torque of the knee extensors in the studies discussed in this section ranged from 60 to 90°, which makes it difficult to draw comparison between studies.

Muscle reaction time, normally measured by assessing the time taken for an individual to respond to an auditory or visual stimulus, can also be used to assess motor performance after eccentric exercise (Linford et al., 2006). In a study from Paschalis et al. (2007) muscle reaction time was assessed by measuring the knee joint reaction angle to release. To do this, the lower limb was passively positioned at four different angles in a random order (0, 15, 30 and 45°). When the limb was relaxed, the investigator released the limb without warning and the angle through which the limb moved before being stopped by the participant was considered the knee joint reaction angle to release. The findings from the study demonstrated that 5 x 15 maximal eccentric contractions of the knee extensors at $60°\cdot s^{-1}$ resulted in an increased knee joint reaction angle at both 0 and 15°. The authors hypothesised that the findings were likely due to sarcomere damage induced by the eccentric exercise bout reducing contraction speed and ultimately the rise in active tension.

The rate of force development (RFD) has been proposed as a more specific and sensitive indirect measure of muscle damage after eccentric exercise than MVIC (Maffiuletti et al., 2016; Peñailillo et al., 2015). RFD is defined as the slope of the force-time curve obtained under isometric contractions, and is a measure of the ability of the neuromuscular system to generate rapid force at the onset of contraction (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002; Blazevich, Horne, Cannavan, Coleman, & Aagaard, 2008). McLellan, Lovell, and Gass (2011) reported significantly decreased peak RFD assessed via countermovement jump (CMJ) both 30 min (≈ -35%) and 24 h (≈ -26%) following a

competitive rugby league match. The authors stated that their results support the notion that an immediate decrease in neuromuscular performance may be related to metabolic disturbances, whilst a decrease at 24 h most likely reflects structural muscle damage and inflammation resulting from exhaustive SSC exercise (Faulkner, Brooks, & Opiteck, 1993).

Isometric squats are another useful model to analyse changes in RFD; Roberts et al. (2015) used this technique to examine acute recovery following high intensity resistance training exercise. Their results showed that from 0-200 ms, RFD was reduced immediately post exercise (≈ -10%) but had recovered to baseline levels by 2 h post. At 4 h post, values were comparable to the immediately post scores. Functional markers were not recorded past 4 h, but these findings support the suggestion from McLellan et al. (2011) that decrements immediately post exercise can be attributed to metabolic disturbances or fatigue, whereas peak functional decrements may occur approximately 24 h post exercise as a result of structural damage and inflammation. McCaulley et al. (2009) also utilised isometric squats to assess functional recovery after strenuous exercise. Their results demonstrated that RFD at 200 ms was attenuated immediately (≈ -30%) and 24 h (≈ -5%) following a high load lower body exercise bout. These findings are supported by Chiu, Fry, Schilling, Johnson and Weiss (2004) and Hakkinen (1994).

Decreases in RFD have been reported following prolonged endurance running (Millet et al., 2002; Petersen, Hansen, Aagaard, & Madsen, 2007), however, data in both studies was collected during evoked, rather than voluntary contractions, and the phase of contraction was not specified. As such, it is not possible to ascertain the extent to which changes in RFD may impact on functional capacity following prolonged running.

The available evidence demonstrates that changes in RFD are evident following strenuous resistance exercise, but that time course and magnitude of change may differ depending on the type of contraction being measured (dynamic versus isometric movements), and the type of training stimulus applied (strength versus hypertrophy) (McCaulley et al., 2009). Furthermore, changes in the early phase of a contraction (<100ms) can normally be attributed to fatigue or other neural factors, whilst changes in the later phase (>100ms) tend to reflect alterations to contractile elements of skeletal muscle (Maffiuletti et al., 2016; Peñailillo et al., 2015), which will in turn influence the time course of recovery. There is currently a paucity of literature examining changes in RFD of voluntary contractions following endurance exercise.

Whilst the majority of literature quantifying recovery post exercise has focussed on MVIC or absolute isokinetic strength losses, RFD is becoming a more popular marker. As already stated, RFD may offer a more sensitive assessment of functional changes, and can be derived easily from sport specific movements. Therefore there is scope to investigate RFD responses using ecologically valid movements following different exercise stresses.

2.3.4. Performance measures

Whilst uniaxial muscle actions measured via dynamometry can offer insight into time courses of recovery following strenuous exercise, these types of movements rarely occur in isolation during human locomotion or sporting movements (Byrne & Eston, 2002). As such, vertical jumping offers a robust model to study performance either with (CMJ or drop jump) or without (squat jump) the influence of the stretch-shortening cycle (SSC). However, SSC-inclusive jumps are often considered more ecologically valid in terms of sports performance (Gathercole, Sporer, Stellingwerff, & Sleivert, 2015).

Large CMJ performance decrements were reported in a study from Chatzinikolaou et al. (2010) who found that 72 h following an intense bout of plyometric exercise (5 x 10 50 cm hurdle jumps and 5 x 10 50 cm drop jumps) CMJ height decreased ~12% in a group of physically active males who were training regularly. Similarly, Abaïdia et al. (2016) utilised an eccentric hamstring protocol to induce damage and reported decrements of ~15% in CMJ performance 24 h post exercise. These findings suggest that eccentric or plyometric based protocols result in large performance decrements, with the greatest reductions seen 24 h post exercise. In contrast, Clifford et al. (2017) investigated changes in CMJ performance following completion of a marathon. Although decrements of approximately 30% were evident immediately post exercise, at 24 h CMJ performance was only reduced by ~5%. A further study from Rousanoglou et al. (2016) reported decrements of only 8% in CMJ height immediately following a mountainous half-marathon race. Although there appear to be fewer studies examining CMJ characteristics following endurance exercise, these results indicate that prolonged endurance exercise, which results in predominantly metabolic damage, has a lesser impact on functional markers such as vertical jumps. The large performance decrements reported immediately post exercise could be attributed to fatigue, defined as an acute and transient failure of a physiological system (such as the SSC) (Abbiss & Laursen, 2005) leading to a failure to maintain the required or expected force or power output (Halson, 2014), rather than functional impairment as a result of structural damage. Clifford et al. (2017) also noted that the magnitude of functional impairment was far lower than in their previous investigation using repeated drop jumps (Clifford, Bell, West, Howatson, & Stevenson, 2016), and was largely absent by 2 days post exercise.

As already mentioned, whilst jump height determined from CMJ is commonly reported, RSI can offer further insight relating to neuromuscular function. Cockburn,

Bell and Stevenson (2013) investigated recovery following completion of 6 x 10 repetitions of unilateral eccentric–concentric knee flexions on a dynamometer at a speed of 1.05rad·s^{-1}. Their results showed that 48 h following completion of muscle damaging exercise, RSI decreased by 18.2 ± 20.1% in the control group. Raeder et al. (2016) investigated the influence of a more ecologically valid 6 day intensified strength training microcycle on markers of fatigue and recovery. Data were reported up to 4 days following completion of the microcycle. Despite CMJ, multiple rebound jump tests and soreness demonstrating a significant return to baseline within the 4 day recovery period, RSI remained significantly reduced. At present, the majority of studies examining acute changes in RSI utilise high intensity intermittent exercise protocols so there is scope to investigate the response following different exercise stresses such as prolonged running or resistance exercise.

2.3.5. Intramuscular proteins

Strenuous exercise can cause muscle tissue to become damaged as a result of mechanical and/or metabolic factors (Brancaccio, Lippi, & Maffulli, 2010). Structural damage to muscle cells can result in increased levels of circulating intramuscular proteins. Although there are a number of skeletal enzymes and proteins discussed in the literature, creatine kinase (CK), myoglobin (Mb) and lactate dehydrogenase (LDH) are some of the most commonly reported markers used to monitor recovery following strenuous exercise. The following sections will focus only on CK as it is the most relevant marker for this thesis.

CK is involved in the conversion of creatine and adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP) within muscle cells. CK is released into the bloodstream following damage or disruption to the cell membrane, and has been shown to present a delayed increase following structural muscle damage in humans (Brancaccio, Maffulli, & Limongelli, 2007; Byrne et al.,

2004). CK is not specific to skeletal muscle and there are at least 5 isoforms known to exist (Brancaccio et al., 2007). When muscle cells become damaged, CK from the intracellular fluid can leak through the cell membrane and into the serum. Cytosolic CK enzymes (found within intracellular fluid) consist of 2 subunits which can be either B (brain type) or M (muscle type); therefore, the 3 different isoenzymes are CK-MM, CK-MB or CK-BB. Normally, only CK-MM is present in the serum, but prolonged and strenuous activity such as marathon running, can result in elevations of all three CK-isoenzymes in the absence of any myocardial damage (Noakes, Kotzenberg, McArthur, & Dykman, 1983). Therefore, when analysing CK to assess damage following strenuous exercise, assays which bind specifically to CK-MM may offer a more accurate characterisation of skeletal muscle breakdown. The ease of sampling and the availability of portable analysers have no doubt contributed to its popularity as an indirect marker of damage. However, the time course of intramuscular protein release is seemingly affected by the intensity, duration and mode of exercise (Brancaccio et al., 2007).

A number of studies utilising high-force eccentric lengthening have demonstrated that CK values increase after exercise and peak (≈ 9 to 46- fold increases) at approximately 3 days post (Chapman, Newton, Sacco, & Nosaka, 2006; Glasgow et al., 2014; Jenkins et al., 2014). However, several studies have employed more sport specific protocols in an effort to ensure their findings are applicable to practice in the field. The majority of these studies have reported a more rapid rise and peak in levels of CK. Hill, Howatson, van Someren, Walshe, and Pedlar (2014), investigated changes in CK following a marathon in recreational marathon runners. Their results showed that CK concentrations were elevated at 24 and 48 hours after exercise, with the highest values occurring at 24 hours (≈ 4 fold increase). Similar results were reported by Hausswirth et al. (2011) who investigated the effect of a number of interventions on recovery following strenuous endurance exercise in highly trained

runners. Participants completed a simulated trail running race including large sections of downhill running on a motorised treadmill. Their results showed that CK was significantly different from baseline until 48 h post, but that peaks occurred 24 hours after completion of the simulated trail run (≈ 2.3-fold).

In terms of resistance exercise, Byrne and Eston (2002) required participants to perform an exercise protocol comprising 10 x 10 barbell squats at 70% body mass load. Their results also demonstrated that CK peaked at 24 hours where values were equivalent to an almost 6-fold change compared to baseline. Similarly, Townsend et al. (2015) reported a 4.5-fold increase in circulating CK 24 h following completion of a heavy load resistance training session (squats, deadlifts and barbell split squats performed at 70-80% 1RM) in resistance trained men.

These studies appear to indicate that peak CK levels are evident earlier following sport specific exercise protocols, compared to repetitive high force eccentric actions of an isolated muscle group (24 h versus 72 h post respectively). This may be due to the larger proportion of active muscle mass utilised during whole body movements such as running and squatting, compared to uniaxial single joint movements. Furthermore, there appears to be a greater magnitude of CK response following resistance, compared to endurance exercise, even in trained individuals.

However, changes in serum CK concentration do not appear to follow the same time course as muscle soreness or muscle function changes following strenuous exercise (Howell et al., 1993). This suggests that while CK measurements can establish that cellular damage has occurred, they cannot be used to measure the magnitude of damage (Fridén & Lieber, 2001). Levels of serum CK only give an indication as to the relationship between the production and removal of the protein following damage (Baird et al., 2012). Similarly, high variability in inter-individual CK responses also makes it difficult to reach a simple conclusion with regards to the

magnitude of damage or injury (Sorichter et al., 1997). Therefore, a number of other blood borne markers, normally pertaining to inflammation, are routinely reported in the literature.

2.3.6. Inflammation

The physiological mechanisms pertaining to the process of inflammation following strenuous exercise are covered in detail in section 2.2. Increased levels of a variety of inflammatory cytokines have been reported following resistance (Phillips, Mitchell, Currie-Elolf, Yellott, & Hubing, 2010; Roberts, Nosaka, Coombes, & Peake, 2015; Vaile, Halson, Gill, & Dawson, 2008), and endurance (Bernecker et al., 2013; Mila-Kierzenkowska et al., 2013; Mündermann et al., 2016; Pournot et al., 2011; Shanely et al., 2013) exercise in humans. A paper from Northoff and Berg (1991) was one of the first to identify IL-6 as a key indicator. Subsequent literature has consistently supported the suggestion that although other cytokines are affected by strenuous exercise, IL-6 appears to demonstrate the greatest acute response. As with the majority of markers affected by strenuous exercise, the magnitude of IL-6 response is modulated by the intensity and duration of the exercise bout.

A review paper from Fischer (2006) reported IL-6 data from a number of studies that had employed knee extensor exercise, cycling or running as an exercise bout. The collated data demonstrated fold increases of up to 189 following high intensity and/or long duration exercise, with a trend for greater magnitudes of disturbance as exercise duration increased. Chatzinikolaou et al. (2010) reported immediate increases in IL-6 following high intensity plyometric exercise (5 x 10 hurdle bounds and 5 x 10 drop jumps). Their results showed that IL-6 was elevated immediately post-exercise (two-fold), and continued to increase until peaking at 24 h post (three-fold). These findings are supported by Phillips et al. (2010) who reported a significant increase in IL-6 following a whole body resistance exercise protocol (≈

1.7 to 3-fold) and Roberts et al. (2015) who documented a sustained increase in IL-6 from 15 min to 2 h (≈ 2-fold) post a high volume squatting protocol performed at 80% 1RM.

A recent study from Della Gatta, Cameron-Smith, and Peake (2014) took repeat biopsy samples before and after a resistance training session and demonstrated a 317-fold increase in IL-6 2 h following three sets of leg press, squat and leg extension at 80% 1RM in untrained men. The large variation in fold increases of IL-6 between the Della Gatta et al. (2014) study and other investigations utilising resistance exercise could be explained by methodological differences relating to sample collection (biopsy versus blood sampling respectively). IL-6 concentrations in contracting skeletal muscle may be 50-100 times higher than those evident in the circulation at any given time (Fischer, 2006). Conversely, Buford, Cooke, & Willoughby (2009) reported no change in serum cytokines 3 h following a high intensity lower body resistance bout despite an mRNA upregulation for TNF-α and IL-6. It is possible that the conflicting results could be attributed to differences in measurement timing. In the study by Buford, Cooke, & Willoughby (2009), post exercise samples were not collected until 3 h after cessation of exercise, meaning that the significant, but transient increase in IL-6 seen immediately following resistance exercise in the other studies, may have been missed.

Similar findings have also been reported following endurance exercise; Bernecker et al. (2013), Mündermann et al. (2016) and Shanely et al. (2013) all reported increases in IL-6 immediately after completion of a marathon run (approximately 15-, 11- and 9-fold respectively). Increases in IL-6 have also been reported following a 48 min high intensity running protocol (16-fold) (Pournot et al., 2011), and a 40 min cycling exercise at ~85% HR_{max} (2.2-fold) (Mila-Kierzenkowska et al., 2013).

The literature cited in the previous paragraphs demonstrates that the time course of IL-6 secretion is comparable following any high intensity exercise bout, irrespective of the exercise modality. Both prolonged endurance exercise and heavy load resistance exercise act as potent stimuli for IL-6 production, exhibiting large but transient increases in the hours immediately post exercise. However, the magnitude of increase appears to be affected by the duration of the exercise bout (Fischer, 2006), with far greater fold increases in IL-6 reported following prolonged endurance exercise compared to resistance training bouts.

CRP, another marker of inflammation is also upregulated in response to increases in circulating IL-6 (Fischer, 2006). Weight, Alexander and Jacobs (1991), Howatson et al. (2010), Mündermann et al. (2016), and Clifford et al. (2017) demonstrated increases in circulating CRP following completion of a marathon, with values peaking 24 h post-race (≈ 21-, 27-, 15-, and 16-fold increases respectively). Mündermann et al. (2016) also stated that marathon finish time accounted for 32% of the variability in serum CRP, with the slowest runners exhibiting the largest increases, and the fastest runners exhibiting the smallest increases.

Increases in circulating CRP have also been reported following plyometric exercise. Chatzinikolaou et al. (2010) examined inflammatory responses following an acute plyometric exercise session. Their findings revealed that CRP peaked 24 h post exercise with a 3-fold increase compared to resting values. Conversely, following resistance exercise in trained individuals, Ingram, Dawson, Goodman, Wallman and Beilby (2009) and Pointon, Duffield, Cannon and Marino (2011) reported no significant changes in levels of circulating CRP. As previously stated, CRP secretion is mediated by IL-6 and it is therefore unsurprising that the magnitude of change in circulating CRP is also affected by exercise duration, with far greater fold changes evident following marathon performance compared to plyometric or resistance based exercise protocols.

Following a marathon, Ostrowski, Rohde, Asp, Schjerling and Pedersen (1999) demonstrated an immediate 2.3-fold increase in TNF-α compared to pre-race values. These results are supported by later studies from Bernecker et al. (2013) and Clifford et al. (2017) who reported immediate increases in TNF-α following completion of a marathon (≈ 1.5 and ≈1.1-fold respectively). Although Bernecker et al. (2013) only compared pre- and post-race values, Clifford et al. (2017) reported that plasma values had returned to baseline levels by 24 h post exercise. In all three studies, changes in TNF-α were of a smaller magnitude than changes to circulating levels of IL-6. Conversely, Mündermann et al. (2016) reported no change in TNF-α following marathon completion. Participants in the Mündermann et al. (2016) study followed a structured 10 week running training programme before completion of the marathon run and therefore may have been more highly trained and/or better accustomed to the race distance despite comparable completion times in both studies. This could explain the observed differences in TNF-α response between studies. Other studies have reported no change in TNF-α following running or cycling protocols where exercise duration was <60 min (Mila-Kierzenkowska et al., 2013; Pournot et al., 2011).

A similar time course of recovery for TNF-α has also been reported following acute resistance training. Townsend et al. (2015) investigated changes in circulating TNF-α in a group of trained males following 4 sets to failure of the squat (80% 1RM), dead lift (70% 1RM), and barbell split squat (70% 1RM). Their results showed a significant increase in circulating TNF-α from baseline to immediately- (≈ 1.3-fold), and 30 min post exercise (≈ 1.2-fold), but that values had returned to baseline by 24 h post. Chatzinikolaou et al. (2010) employed 100 plyometric actions to induce damage and measured CRP responses up to 120 h post exercise. Their results showed that plyometric exercise caused a marked increase in CRP at 24 h post (≈ 3-fold), but that values returned to baseline levels by 48 h.

The magnitude of TNF-α response following strenuous exercise appears to be smaller than other inflammatory markers such as CRP and IL-6, regardless of exercise mode. Whilst small increases have been reported following resistance exercise, changes are not always evident following endurance exercise in trained individuals.

In summary, a number of cytokines are upregulated following strenuous exercise, and the magnitude of change appears to be dependent upon exercise duration. Typically, greater increases in IL-6 and CRP are reported following prolonged endurance exercise compared to resistance exercise. Irrespective of exercise mode, TNF-α and IL-6 appear to peak immediately post exercise, whilst CRP continues to rise until 24 h post. There is some evidence demonstrating small increases in TNF-α following both resistance and endurance exercise, although training status may influence the magnitude of response following endurance exercise. Whilst markers of inflammation are useful to establish the presence of structural muscle damage, the magnitude of change and time course of recovery may not necessarily coincide with functional decrements and subsequent recovery. Furthermore, whilst the inflammatory response post-exercise has been extensively researched, and it is known that inflammation is an integral part of muscle repair and adaptation, the 'optimal amount' of inflammation has yet to be quantified.

2.3.7. Summary

The literature discussed within the previous few sections demonstrates that strenuous exercise, irrespective of mode, can elicit perturbations in a number of perceptual, performance and physiological markers. However, the magnitude of change, and time course of recovery differs between markers and is also dependent upon the exercise stress applied, highlighting the need to approach recovery research from a holistic perspective. Furthermore, the differing responses following

endurance and resistance exercise demonstrates a need to assess the effectiveness of any recovery intervention after different exercise modalities.

2.4. Adaptation following strenuous exercise

Whilst the previous sections of this review focused on the potential negative impact of strenuous exercise on subsequent exercise performance, it is important to understand the interrelationship between the process of muscle breakdown and the resultant adaptive responses. The majority of exercise induced adaptations to training take place during the recovery period (Bishop, Jones, & Woods, 2008). It follows that training adaptations are achieved relative to the stimulus applied, and as such the specificity of training prescription is considered the fundamental foundation of any training programme (Minett & Costello, 2015). Endurance training is primarily used to improve cardiovascular parameters such as maximal oxygen uptake, arterial O_2 capacity, cardiac output, stroke volume, resting heart rate and arteriovenous O_2 difference (Andrew, Guzman, & Becklake, 1966; Ekblom, 1969; Hanson, Tabakin, Levy, & Nedde, 1968; Sagawa, 1978). However, the final study of this thesis investigated the potential influence of recovery strategies on adaptations to strength and power training, therefore this section will focus on the mechanisms underlying adaptations to resistance training.

2.4.1. Responses to resistance training

Resistance training results in increased force generating capacity (strength), due to neural adaptations leading to enhanced effectiveness of muscular coordination (Carroll, Riek, & Carson, 2001), as well as muscle hypertrophy (increased lean muscle mass) (Broatch, Petersen, & Bishop, 2018; Hedayatpour & Falla, 2012; Maffiuletti et al., 2016). Early adaptations to resistance training are thought to be primarily explained by neural changes, with a greater contribution from

morphological changes as training progresses (Buckthorpe, Erskine, Fletcher, & Folland, 2015).

It has been established that hypertrophy occurs as a consequence of increased muscle fibre size due to increases in protein synthesis and the addition of structural elements such as myofilaments, myofibrils and sarcomeres (Frontera & Ochala, 2015). It has been well established that the ingestion of dietary protein after resistance exercise inhibits muscle protein breakdown and stimulates an increase in muscle protein synthesis rates, resulting in net muscle protein accretion during the acute recovery phase (Cermak, Res, de Groot, Saris, & van Loon, 2012). Therefore, dietary protein supplementation is often considered necessary in order to maximise the adaptive response of skeletal muscle to any structured resistance training programme (Gillen et al., 2017; Heaton et al., 2017; Phillips & Van Loon, 2011). As already discussed, although muscle fibres demonstrate incredible elastic properties, repeated contractions can result in structural damage (Armstrong et al., 1991; Cleak & Eston, 1992; Faulkner et al., 1993). However, as outlined in figure 2.1, the final stage in the process of exercise induced muscle damage is regeneration and repair. During this final phagocytic phase, the stimulation of satellite cells to divide is likely to be a crucial process (Kendall & Eston, 2002) that precipitates the addition of new myonuclei.

2.4.2. Molecular pathways and signalling molecules

There are several factors and molecular pathways that regulate muscle mass regulation and the hypertrophic response (Russell, 2010), and these pathways are activated after a bout of resistance exercise (Mayhew, Kim, Cross, Ferrando, & Bamman, 2009). Increases in muscle protein synthesis are largely attributed to the upregulation of protein translation initiation and control by mammalian target of rampamycin (mTOR). mTOR pathways can be activated by a number of stresses or

intracellular changes including, but not limited to, mechano-stimulation, alterations in calcium activity, increases in ROS, increases in cytokines (IL-6 in particular), or increases in insulin-like growth factor-1 (IGF-1).

Satellite cells are present between the basal lamina and sarcolemma and act as 'myogenic stem cells' (Schoenfeld, 2010). Although normally in an inactive or dormant state (Vierck et al., 2000), once stimulated, satellite cells proliferate and fuse to existing cells or each other to create new myofibres, creating the precursors required for repair and regeneration (Toigo & Boutellier, 2006). There are a number of mechanisms or pathways by which satellite cell signalling occurs. It appears that macrophage infiltration is an essential prerequisite for regeneration and may stimulate satellite cell proliferation in the first instance (Kendall & Eston, 2002; Lescaudron et al., 1999; Merly, Lescaudron, Rouaud, Crossin, & Gardahaut, 1999). However, satellite cells can also be stimulated via IGF-1, IL-6 or mTOR, and the effects are molecularly transduced to downstream targets that alter muscle protein balance in favour of synthesis rather than degradation (Schoenfeld, 2010).

One of the key pathways is the Akt/mTOR pathway, where IGF-1 binds to receptors on the sarcolemma and induces the activation of the Akt protein. Although the specific molecular mechanisms have not yet been fully elucidated (Schoenfeld, 2010), Akt is considered both an effector of anabolic signals, and a dominant inhibitor of catabolic signals (Toigo & Boutellier, 2006).

2.4.3. Role of protein breakdown and synthesis in the hypertrophic response

As previously stated strenuous exercise leads to increased intracellular Ca^{2+}, increased ROS, and elevated levels of certain inflammatory cytokines, all of which are implicated in molecular signalling pathways related to hypertrophy. As such, the process of muscle protein breakdown and subsequent repair may be necessary in order for hypertrophy to take place. As already discussed, IL-6 is a key marker of

acute inflammation following exercise (Calle & Fernandez, 2010; Fischer, 2006; Zhang & An, 2007), and has also been shown to promote anabolism (Toigo & Boutellier, 2006; Vierck et al., 2000). Similarly, a number of calcium dependant pathways have also been implicated in the regulation of muscle hypertrophy (Schoenfeld, 2010). The mitogen-activated protein-kinase pathway (MAPK) is considered a key regulator of gene expression and metabolism (Kramer & Goodyear, 2007) and has also been shown to link cellular stress with an adaptive response in myocytes, modulating growth and differentiation (Roux & Blenis, 2004). It is also important to acknowledge the integral role that hormones and cytokines play in the hypertrophic response, acting as upstream regulators of anabolic processes (Schoenfeld, 2010).

An investigation from Damas et al. (2016) suggests that increased myofibrillar protein synthesis evidenced after a bout of resistance exercise is initially used for muscle repair before being directed to support muscle fibre hypertrophy, but that muscle damage does not play a role in skeletal muscle hypertrophy during prolonged resistance training. A follow up study from the same group Damas, Libardi and Ugrinowitsch (2018) also concluded that increased satellite cell activity during a 10 week resistance training programme was more important for repair than hypertrophy. However, a recent review from Schoenfeld, Ogborn, Vigotsky, Franchi, and Krieger (2017) states that although researchers speculate that skeletal muscle damage mediates an anabolic response, a lack of studies investigating this relationship means that the role of muscle damage in hypertrophic adaptations remains unclear.

In summary, resistance training leads to increases in strength, alterations in neuromuscular function and increases in lean muscle mass as a result of hypertrophy. Whilst the acute physiological responses to strenuous exercise (disruption of calcium homeostasis, swelling and inflammation) may be detrimental

in the short term, it is possible that the processes are necessary as part of the regenerative phase of muscle damage, in order to initiate molecular pathways that lead to hypertrophic adaptations. As such, where recovery interventions are employed to attenuate oedema, inflammation and secondary damage post exercise it is possible that adaptive responses may be attenuated over time.

2.4.4. Evidence of extracellular remodelling

The success of the regenerative phase of muscle fibre breakdown and repair is determined not only by the activation of satellite cells, but also by the level of collagen deposition in the extracellular matrix (Vieira Ramos et al., 2016). Whilst alterations in the extracellular matrix are routinely examined using histological techniques, it is possible to assess physiological adaptations to training without the need for biopsy samples. Procollagen I N-terminal peptide (PINP) and Procollagen III N-terminal peptide (PIIINP) are markers of bone and collagen turnover respectively. Although PINP is most abundant in bone, it is also present in soft connective tissues and circulating concentrations can provide an accurate reflection of the synthesis rate of collagen type I (Jensen, Johansen, Skovsgaard, Brandt, & Teisner, 2002). Similarly, PIIINP in the circulation can be derived from the synthesis and deposition of new type III collagen fibrils in muscle (Berry et al., 2013).

Although both sensitive markers of bone and collagen formation, PINP and PIIINP are not commonly reported within sport science literature, and are used more routinely for the assessment of clinical pathologies. Despite some recent longitudinal studies showing no significant change following endurance (Cornelissen, Fagard, & Lijnen, 2010; Zanker & Swaine, 2000) or resistance training (Bloomquist et al., 2013; Sartorio et al., 2001), a study from Evans et al. (2008) demonstrated that 4 months of military combat training resulted in increased circulating levels of PINP, and that these changes were evident from 2 months. Similarly, Raastad et al.

(2010) reported that a single session of high-force eccentric exercise was sufficient to upregulate PIIINP, suggesting that PIIINP is therefore a suitable marker to assess remodelling of the extracellular matrix after exercise. Therefore, inclusion of markers such as PINP and PIIINP in future adaptation research could offer insight into the mechanisms underpinning physiological and performance changes resulting from a structured training programme.

2.4.5. The potential role of sleep

Another important aspect that influences both physiological and cognitive factors is sleep. A reduced quality or quantity of sleep can have a significant effect on athletic performance as well as learning, cognition, pain perception and inflammation (Halson, 2014b; Nédélec, Halson, Abaidia, Ahmaidi, & Dupont, 2015). A study from Venter (2012) demonstrates that both athletes and coaches consider sleep as a vital part of the recovery process. Whilst sleep polysomnography is considered the gold standard for sleep assessment, it can be expensive and labour intensive and is therefore normally reserved for the assessment of clinical sleep disorders (Sadeh, 2011). An alternative method of sleep assessment is through the use of self-report diaries either independently, or in conjunction with actigraphy.

Previous research has demonstrated that partial sleep deprivation can negatively impact upon reaction time and attentional capacity (Jarraya, Jarraya, Chtourou, & Souissi, 2013; Taheri & Arabameri, 2012), submaximal resistance exercise performance (Reilly & Piercy, 1994) and inflammation post exercise (Abedelmalek et al., 2013). Whilst extended sleep deprivation is unusual for athletes, the time demands of training, competition and post training recovery interventions can result in sleep disturbance or decreases in total sleep time. Currently, there appears to be a lack of research examining the impact of recovery interventions on sleep over a prolonged period of time. As such there is scope to examine whether post exercise

recovery interventions can enhance sleep quality, and ultimately influence adaptation.

2.4.6. Summary

From the information presented in this, and previous sections, it is clear that although considered detrimental in the short term, muscle damage and inflammation are necessary processes required for adaptation to occur. The use of recovery interventions to attenuate any disadvantageous effects of strenuous exercise is almost universal in competitive sport (Barnett, 2006; Halson, 2013; Vaile, Halson, & Graham, 2010; Venter, 2012), but the specificity of recovery interventions and their potential long term impact warrant further investigation.

2.5. Cryotherapy as a recovery strategy

Given the wealth of research demonstrating the potential negative impact of strenuous exercise on a number of physiological, perceptual and performance markers, it follows that the rate and quality of recovery is extremely important and that optimal recovery may provide numerous benefits during repetitive high-level training and competition (Gill, Beaven, & Cook, 2006). As already discussed, differences in the mode, duration and intensity of exercise influence the time course and magnitude of symptoms in the days following exercise. It follows that different recovery strategies may be suitable following different exercise stresses, therefore, investigating different cryotherapy interventions and their effect on recovery and performance following different exercise modalities is important (Minett & Costello, 2015).

As already indicated, recovery strategies are predominantly used to accelerate recovery time between successive bouts of exercise and in that sense could be described as acute treatments. However, when used regularly, there is scope for acute benefits to diminish longer term adaptations. Therefore, there is a need to

examine the use of any recovery intervention after exercise, and examine the potential for any maladaptive responses which could be detrimental to longer term performance gains. The following section of this review will discuss the impact of acute cryotherapy application on markers of recovery following strenuous exercise, as well as literature relating to the effect of repeated exposures on training adaptations. As already alluded to, whilst the term cryotherapy can be broadly applied, this review will focus on CWI and WBC as these are the two modalities examined within this thesis. For more information regarding other forms of cryotherapy please refer to the following reviews: Bieuzen et al. (2013) (contrast bathing); Bleakley et al. (2004) (ice packs, crushed ice & commercial icing equipment).

2.5.1. Mechanisms of cryotherapy

A number of mechanisms have been proposed in an attempt to explicate reported physiological changes following cryotherapy exposure (Swenson, Swärd, & Karlsson, 1996). Figure 2.4 outlines some of the key mechanisms associated with CWI that may impact on recovery. It is likely that WBC elicits many of the same responses, but there is no influence of hydrostatic pressure. Changes in physiological mechanisms resulting from a decrease in muscle and/or skin temperature include; analgesia, reductions in cardiovascular strain, decreased blood flow, reduced tissue metabolism, increased removal of muscle metabolites, reduced oedema as well as neuromuscular, cardiovascular and hormonal changes (Ihsan, Watson, & Abbiss, 2016; Leeder, Gissane, van Someren, Gregson, & Howatson, 2012). It is likely that any therapeutic effects resulting from a cryotherapy intervention could be attributed to one, or a combination, of these physiological phenomena.

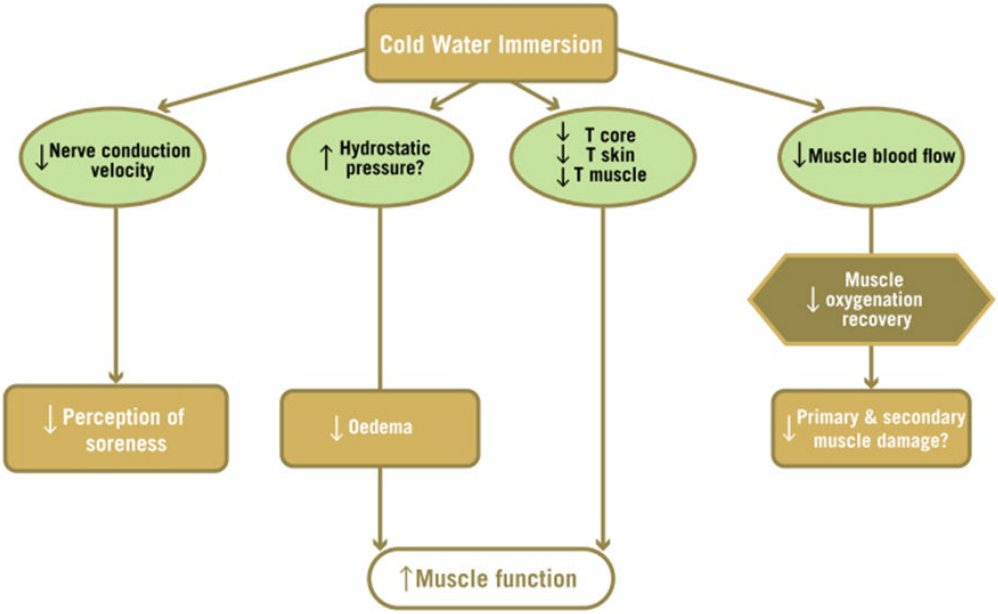

Figure 2.4. Suggested mechanisms by which CWI improves recovery from EIMD. From McGorm et al., 2015.

During exercise, body temperature increases (Fortney & Vroman, 1985; Gisolfi & Wenger, 1984; Gleeson, 1998) and blood is redirected from active musculature to the cutaneous circulation for heat dissipation and temperature regulation (Ihsan et al., 2016). This shift in blood flow to the peripheries results in an increased cardiovascular strain characterised by a decreased central blood volume leading to reduced muscle blood flow and an impairment of oxygen delivery (González-Alonso & Calbet, 2003). Cryotherapy application is utilised in order to modulate temperature and blood flow alterations, and to minimise any potential deleterious effects of these physiological responses.

Skin temperature drops rapidly in the first 1-3 minutes of CWI and WBC exposure and reaches minimum temperatures after approximately 8-9 minutes (Janwantanakul, 2009). Superficial muscle tissues cool more rapidly than deep tissues, but deeper tissues will often reach minimum temperatures after cooling ends, as heat is lost to the colder, more superficial tissues. As such, the use of

intermittent cooling strategies can allow muscle to reach colder temperatures whilst minimising the risk of damage to the skin, as muscle tissue continues to cool during the post-application period, and skin will re-warm rapidly (White & Wells, 2013).

Vasoconstriction is a homeostatic mechanism that occurs in response to a drop in temperature in an effort to prevent heat loss (Sawka & Young, 2006). The rapid drop in skin temperature facilitated by CWI or WBC exposure results in cutaneous vasoconstriction, redirecting blood back to the central circulation thereby reducing cardiovascular strain (Ihsan et al., 2016). The redirection of blood from the muscle to the central circulation is also suggested to accelerate the removal of muscle metabolites, improving metabolic recovery following intense bouts of exercise (Hausswirth et al., 2012; Pointon, Duffield, Cannon, & Marino, 2012). The potential effect of hydrostatic pressure may offer a further mechanism for the increased removal of muscle metabolites after CWI. However, whilst cryotherapy may facilitate a shift in blood volume from the periphery to the central circulation, it may also result in a concomitant decrease in muscle blood flow. As already suggested, a decrease in muscle blood flow may lead to an impairment of oxygen and nutrient transport and utilisation which could ultimately be detrimental to recovery (Ihsan et al., 2016).

Cold-induced vasoconstriction is also thought to reduce the permeability of cellular, lymphatic and capillary vessels which in turn diminishes fluid leakage following structural damage to muscle fibres (Leeder et al., 2012). More recent studies have investigated the impact of cryotherapy interventions on blood flow (Mawhinney et al., 2013, 2017), stating that vasoconstriction is increased and femoral artery conductance is decreased following exposure. Further, the latter study concluded that blood flow is mediated to a greater extent following CWI compared to WBC, likely as a result of the differences in thermal input (e.g. core and local tissue temperatures) between conditions. The mediation of blood flow evidenced in these studies is likely to further impact on the inflammatory cascade and migration of

leucocytes (Mawhinney et al., 2017). This decrease in fluid migration has been proposed as the main mechanism responsible for reducing inflammation following strenuous exercise (Mawhinney et al., 2017). Evidence suggests that local vasoconstriction and decreased perfusion can persist for longer than cryotherapy is applied (Khoshnevis, Craik, & Diller, 2015). Consequently, cold applications greater than 30 minutes in duration have been shown to increase oedema due to permanent cold damage to muscle cells (Khoshnevis et al., 2015; Weston, O'Hare, Evans, & Corrall, 1987). Whilst the blunting of the inflammatory process may seem counterintuitive for recovery, muscle fibres with sarcolemmal disruption or increased osmolality have an increased risk of oedema (White & Wells, 2013). Therefore, reducing blood flow is thought to decrease swelling and subsequent pain in muscle fibres, functional impairment and the potential for further damage caused by the secondary phase of inflammation.

Following exercise, energy demand in stressed muscle fibres can increase in order to accommodate repair to structural damage and the restoration of depleted energy stores (Hom, Vasquez, & Pozos, 2004). As already discussed, the respiratory burst can lead to greater production of ROS as a result of neutrophil activation (Dahlgren et al., 2007). Research has demonstrated that ROS production from activated neutrophils is inhibited at temperatures below 37°C (Catenaccio et al., 1999). As such, where cryotherapy application leads to a reduction in muscle temperature, ROS production may be attenuated which could in turn limit ROS mediated secondary damage (White & Wells, 2013).

As well as localised alterations to temperature, blood flow and swelling, cryotherapy can also alter endocrine, cardiovascular and neuromuscular responses. Cold exposure can affect sensory neurons, which in turn can reduce perceptions of pain associated with swelling (Gregson et al., 2011; Herrera, Sandoval, Camargo, & Salvini, 2010). However, cold can also be interpreted by the body as stress, and a

number of physiological and immune responses arise as a result of exposure. Physiological adjustments are brought about by the stimulation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. As already discussed, one well documented physiological response is increased peripheral vasoconstriction. Vasoconstriction helps maintain core body temperature by reducing blood flow, and therefore heat loss to the skin, and decreasing the thermal gradient between the skin and the external environment (LaVoy, McFarlin, & Simpson, 2011). In addition to these 'useful' transient responses, stimulation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis also results in hormone release, including epinephrine, norepinephrine and the stress hormone cortisol. Even partial exposure in ≤10° water, such as hand or foot immersion, will cause an increase in norepinephrine and cortisol (Houben, Thien, Wijnands, & Van't Laar, 1982). In terms of immune responses, an increased production of corticosteroids and catecholamines following cold exposure can impact upon immune cell function. This increased hormone response can induce leucocytosis, reduce the production of inflammatory mediators, decrease the expression of adhesion molecules on inflammatory cells and are associated with a decrease in the lymphoproliferative response, which is a measure of immune function (LaVoy et al., 2011). It is worth noting that leucocytosis was not observed after a 40 min exposure in 23°C water, but was evident after exposure in 14°C water (Cross, Radomski, Vanhelder, Rhind, & Shephard, 1996; Jansky et al., 1996), suggesting that immunological parameters, much like physiological changes, are dependent on the severity of cold exposure. Therefore, when utilising any cryotherapy intervention, there is a need to achieve equilibrium between optimising therapeutic benefit and minimising any potential detrimental impact of a stress related response.

The impact of any cryotherapy intervention is largely dependent on its capacity to affect a change in skin or muscle temperature, therefore the physical characteristics

of participants can influence results. A recent paper by Hammond, Cuttell, Nunley and Meyler (2014) found that individuals with different anthropometric characteristics received different therapeutic effects from cryotherapy in relation to the magnitude of skin cooling following exposure. Their results suggested that individuals with higher levels of adiposity experienced greater cooling than thinner individuals, and that for a given fat free mass index females cooled more than males. Cryotherapy elicits a comparable pattern of linear cooling in skin, superficial tissue and deep tissue, despite differences in the rate of cooling (Costello, Algar, & Donnelly, 2012). It is worth noting that whilst skin temperature drops rapidly during cold exposure (Janwantanakul, 2009), deeper tissues such as muscle cool much more slowly. Whilst Hammond et al. (2014) suggest that higher adiposity results in a greater magnitude of skin cooling, other studies indicate that increased adiposity actually slows intramuscular temperature changes (Jutte, Merrick, Ingersoll, & Edwards, 2001; Otte, Merrick, Ingersoll, & Cordova, 2002). It is likely that decreases in intramuscular temperature would result in greater attenuation of the inflammatory response compared to superficial skin cooling, meaning that leaner individuals would likely experience a greater therapeutic benefit (Stephens et al., 2016). Body fat has a low heat conductivity meaning that it provides greater insulation and thermal resistance than either skin or muscle (Stephens et al., 2016). Similarly, blood flow can also be impacted by body composition; individuals with higher adiposity tend to demonstrate a lower than average blood volume per unit weight (Zhang, Huizenga, Arens, & Yu, 2001). This in turn impacts conductive heat transfer through the tissues, improving the insulating capacity of individuals with greater body fat (Stephens et al., 2016). It is clear from these studies that differences in anthropometrics have the potential to impact upon the efficacy of cryotherapy interventions following recovery.

2.5.2. Acute responses to cryotherapy

The results of studies investigating acute responses to cryotherapy following strenuous exercise (Table 2.1) are equivocal. The majority of studies show improvements in one or more markers of recovery (Bailey et al., 2007; Hausswirth et al., 2011; Ingram et al., 2009; Montgomery et al., 2008), whilst others show no benefit (Costello, Algar, & Donnelly, 2012; Leeder et al., 2015). The studies implement a variety of methodologies (exercise protocols; markers of recovery; timing of measurements; study design; cryotherapy modality; timing of exposure), which could explain some of the dissimilarity seen in the reported results.

Table 2.1. Studies reporting the effects of cryotherapy on acute recovery

Study	Exercise protocol	Cryotherapy intervention	Effects post intervention
CWI			
Bailey et al., 2007	90min intermittent shuttle running	10min CWI at 10°C Non-immersion control	DOMS ↓ at 1, 24 & 48h* MVIC ↑at 24 and 48h* CK↔* Mb↓ at 1h*
Montgomery et al., 2008	3 day basketball tournament	5 x 1min CWI at 11°C separated by 2min CHO + stretching	Line drill performance↑* 20m sprint (s) ↓* Agility↔* Vertical Jump ↑* Flexibility↑* DOMS↓* Fatigue↓*
Vaile et al., 2008	5 x 10 eccentric bilateral leg press at 120% of concentric 1RM followed by 2 x 10 at 100% 1RM	4 x 14min CWI at 15°C applied over 4 days Passive recovery	Isometric squat ↑ at 48 & 72h* Weighted squat jump ↑ at 48h* Mid-thigh girth ↓at 24, 48 & 72h* CK ↓ at 24 & 72h* Mb, IL-6 or LDH ↔ * DOMS ↔ *
Ingram et el., 2009	80min exhaustive simulated team sport exercise	2 x 5min CWI at 10°C separated by 2.5min Control	DOMS↓ at 24h* MVIC ↔at 48h[b] Total sprint time (s)↓at 48h* CK↔* CRP↔*
Ascensao et al., 2011	90min soccer match	10min CWI at 10°C Thermoneutral immersion	CK ↓ at 24 and 48h* Mb ↓at 30min* CRP ↓at 30min and 24h* Sprint ability ↔*[b] CMJ ↔* Quad strength ↑at 24h* DOMS ↓in quads at 24h*

Pournot et al., 2011(a)	2 x 10min exhaustive intermittent exercise comprising CMJ and rowing	15min CWI at 10°C Passive recovery	MVC ↑at 1h* CMJ ↑at 1h* CK ↓at 24h* Leucocytes, neutrophils and monophils ↓at 1h*
Broatch et al., 2014	4x30s max sprints against a load corresponding to 7.5% body weight on cycle ergometer	15min CWI at 10.3 ± 0.2° Thermoneutral immersion	MVC_{peak} ↔*[a] Physical readiness for exercise ↑ at 24h*[a] vigour ↑*[a] IL-6↔*[a] Thigh girth↔*[a] Algometry↔*[a] Muscle temperature ↓*[a]
Ihsan et al., 2014	30min continuous running at 70% $\dot{V}O_2$ max, followed by intermittent running to exhaustion at 100% $\dot{V}O_2$ max	15min unilateral lower limb CWI at 10°C Contralateral limb served as control	Muscle temperature ↓immediately post* PGC-1α↑at 3h* mRNA ↑at 3h*
Roberts et al., 2014	6 sets of front- and back-squats at loads corresponding to 8-, 8-, 10-, 12-, 10- and 10-RM; 3 x 12 walking dumbbell lunges with a load corresponding to 40% of body mass; 3 x 12 DJ from a height of 50cm.	10min CWI at 10°C 10min low intensity self -paced cycling	Maximal muscle function recovery time↔* Submax muscle function↑6h* BLa↑ at 2h* Venous O_2 saturation ↓ at 2h* Mb↓* ↔Serum endothelin-1 concentration (↓in active recovery) DOMS ↓at 5h*
White et al., 2014	12 x 120m sprints with 3min rest between sprints	10min CWI at 20°C 30min CWI at 20°C 10min CWI at 10°C 30min CWI at 10°C	IL-6↑ at 1.5 and 2h* IL-6↓ at 2h in 10 x 20°C only IL-8↑ immediately post in all conditions EXCEPT 10 x 20°C squat jump height ↓ at 48h for 10 x 20°C and 30 x 10°C soreness↔

			perceived impairment↔ Drop jump ↓ at 24 and 48h in CON and 10min × 20°C only
Leeder et al., 2015	LIST	14min CWI at 14°C Control	DOMS↔*
Roberts et al., 2015	10 x 20 maximal knee extensions at 90°·sec^{-1}	10min CWI at 10 ± 0.2°C Active recovery	Systolic BP↑* HR ↓at 50-70min* Muscle oxygenation↔* Muscle and skin temp↓* Isometric PT ↑at 5, 20 and 40min*
Barber et al., 2017	Simulated rugby match	2 x 5min CWI at 10°C separated by 2.5min Control	DOMS ↓ at 24 and 48h* CMJ ↑* MVIC ↑* CK ↓*
WBC			
Hausswirth et al., 2011	48min simulated trail run	3min WBC at -110°C Passive recovery	DOMS↓ at 1h* Tiredness↓ at 1h* MVIC ↑ at 1h* CK↔* Wellbeing ↑ at 24h*
Pournot et al., 2011(b)	48min simulated trail run	4 x 3min WBC at -110^{0}C applied over 4 days (1 session/day) Passive recovery	TNF-α at any time point* IL-6↔* IL-10↔* IL-1ß ↓ at 1h* IL-1ra ↓at 24h* CRP ↓ at 1-96h* Leucocyte count↔*
Costello et al., 2012	5 x 20 eccentric actions of the quadriceps at 1.57rads·s^{-1}	2 x 3min WBC at -110°C applied 24 h post exercise 2 x 3min WBC at 15°C applied 24h post exercise	MVIC ↔* DOMS↔*

Fonda & Sarabon, 2013	5 x 10 drop jumps and 5 x 10 bilateral leg curls at 75% Concentric 1RM	7 x 3min PBC at -140 to -195°C applied over 7 days (1 session/day) Control	DOMS↓ from 1-72h* RTD ↑at 24h* CK, AST, LDH ↔*
Markovic et al., 2014	Plyometric exercise	5 x 3min PBC at –140 to –195°C applied over 5 days (1 session/day) Control	↑RTD* ↓pain*
Selfe et al., 2014	Competitive rugby league match	1, 2 or 3min WBC at -135^{0}C	IL-6↔ in any group Deoxyhaemoglobin ↓for gastroc and VL Oxyhaemoglobin and tissue oxygenation↓for VL Core temperature↔ Thermal sensation and comfort↓
Ferreira-Junior et al., 2015	5 x 20 drop jumps	3min PBC at -110°C Control	Isometric PT of knee extensors↑ at 72 and 96h* Muscle thickness ↓at 24 and 96h* DOMS ↓at 72h*
Krüger et al., 2015	5 × 5min running at 90% of max velocity. Ramp-test to exhaustion pre and post (R1 & R2)	3min WBC at -110^{0}C 1h passive rest at ~22°C	Difference in time to exhaustion between R1 and R2↓* O2 content of VL↑* Submax $\dot{V}O_2$ ↓* Submax HR↓* Submax RPE↓*
CWI vs WBC			
Abaïdia et al., 2016	5 x 15 single leg eccentric hamstring action	10min CWI at 10°C OR 3min WBC at -110°C	Single leg CMJ ↑ at 72h compared to WBC CMJ ↑ at 72h compared to WBC DOMS ↓ at 48h compared to WBC

Hohenauer et al., 2017	5 x 20 drop jumps	10min CWI at 10°C OR PBC −60°C for 30sec, −135°C for 2min	Perception of recovery ↑ at 24h compared to WBC Cutaneous vascular conductance ↓ at 30min compared to PBC Thigh muscle O_2 saturation ↓ at 40min compared to PBC Thigh/shin skin temp ↓ at 1h compared to PBC DOMS ↔ between groups MVIC ↔ between groups CMJ ↔ between groups

1RM=1 Repetition Maximum, AST=Aspartate Aminotransferase, BLa=Blood Lactate, BP=Blood Pressure, CHO=Carbohydrate, CK=Creatine Kinase, CMJ=Countermovement Jump, CON=Control, CRP=C-reactive protein, CWI=Cold Water Immersion, DJ=Drop Jump, DOMS=Delayed Onset Muscle Soreness, HR=Heart Rate, IL=Interleukin, LDH=Lactate Dehydrogenase, LIST=Loughborough Intermittent Shuttle Test, Mb=Myoglobin, mRNA=Messenger Ribonucleic Acid, MVC=Maximal Voluntary Contraction, MVIC=Maximal Voluntary Contraction, O_2=Oxygen, PBC=Partial Body Cryotherapy (head not exposed), PGC-1α= Peroxisome Proliferator-activated Receptor Gamma Coactivator 1-alpha, PT= Peak Torque, RPE=Rate of Perceived Exertion, RTD= Rate of Torque Development, TNF-α=Tumour Necrosis Factor α, VL=Vastus Lateralis, $\dot{V}O_2$=Volume of Oxygen, WBC=Whole Body Cryotherapy, ↑=indicates higher values, ↓=indicates lower values, ↔=indicates no difference between groups; *=compared to control , [a]=compared to placebo, [b]=compared to baseline

2.5.2.1. Muscle soreness

Muscle soreness is one of the most common symptoms reported by athletes following strenuous exercise. Consequently, perceptions of muscle soreness are one of the most frequently reported outcome measures in the literature. A number of mechanisms attributed to cryotherapy have the potential to minimise acute muscle soreness following strenuous exercise.

Cryotherapy can reduce the osmotic pressure of escaped fluid, thereby reducing the pain response (Gregson et al., 2011). There is also evidence to suggest that CWI can reduce sensations of pain and reflective spasm by reducing the conductive velocity of both sensory and motor neurons respectively (Herrera et al., 2010). Whilst neurons display similar cooling patterns to skin and muscle tissues, sensory neurons appear to be influenced by smaller temperature changes than motor neurons, possibly due to their more superficial anatomic location (Merrick, Knight, Ingersoll, & Potteiger, 1993). Both of these mechanisms mean that cryotherapy has the potential to limit soreness immediately following strenuous exercise resulting in improved perceptions of wellbeing and recovery. A recent review from Rose et al. (2017) suggests that WBC can also offer analgesic benefits compared to a control condition.

Both CWI (Ascensão et al., 2011; Bailey et al., 2007; Roberts et al., 2014) and WBC (Ferreira-Junior et al., 2015; Fonda & Sarabon, 2013; Hausswirth et al., 2011; Markovic, Fonda, & Nejc, 2014) have been shown to attenuate soreness following strenuous exercise compared to a control group. The studies mentioned here utilised plyometrics, heavy resistance training, or long duration endurance running or cycling as the initial exercise bout.

However, some studies have reported no difference in soreness compared to a control group following strenuous exercise. Despite using the same exercise

protocol as Bailey et al. (2007), Leeder et al. (2015) reported that there was no significant difference between seated CWI and a control group after completion of the Loughborough Intermittent Shuttle Test (LIST). The authors suggested that their contrasting findings may have been due to differences in the CWI protocol; Bailey et al. (2007) used a 10 min exposure at 10°C, whilst Leeder et al. (2015) used a 10 min at 14°C exposure. Whilst neither study reported measures of muscle or skin temperature, it may be that lower intramuscular temperatures were achieved in the Bailey et al. (2007) study. As already discussed, the ability to successfully cool muscle fibres underpins the majority of mechanisms associated with cryotherapy.

In terms of WBC, Costello, Algar and Donnelly (2012) reported that WBC (2 x 3 min) at -110°C following 100 high-force maximal eccentric contractions of the left knee extensors was not sufficient to attenuate perceived soreness scores up to 72 hours post exercise compared to a control group. The finding that WBC was ineffective at attenuating DOMS is in contrast to the findings from Fonda and Sarabon (2013), Markovic et al. (2014) and Ferreira-Junior et al. (2015). The conflicting results could be due to the timing of the exposure; whereas most investigations utilise WBC immediately post exercise, participants in the Costello et al. (2012) study were not exposed to treatment until 24 hours after completion of the exercise bout, potentially missing the optimal 'window of opportunity' (Luttrell & Halliwill, 2015). This theory is supported by findings from Pournot et al. (2011b) who demonstrated that WBC can effectively reduce the inflammatory process following a simulated trail run, (evidenced by decreases in IL-1β (1h post) and CRP (24h post)) compared to a passive recovery group. The initial inflammatory process begins immediately following strenuous exercise or injury (Nadler et al., 2004; Ostrowski et al., 1999; Tidball, 2005; Toumi & Best, 2003), and as such, when applied 24 hours after an exercise bout, cryotherapy is likely to be ineffective at preventing or reducing

symptoms associated with the natural inflammatory response, such as increased soreness and strength decrements.

Two further studies by Broatch, Petersen and Bishop (2014) and White, Rhind and Wells (2014) also reported no difference in muscle soreness between groups following 4 x 30 second maximal sprints on a cycle ergometer and 12 x 120 m sprints on foot respectively. Broatch et al. (2014) examined the effects of CWI on recovery following 4 maximal intensity sprints on a cycle ergometer, and at the time of writing, it appears to be the only paper that has used a purely metabolic exercise stress to examine the effects of CWI on recovery. It is possible that methodological differences could explain these contrasting findings. The use of a predominantly metabolic exercise stress could mean that soreness normally associated with structural damage and micro trauma may not have been present but that increases in metabolites or ROS could be responsible for soreness (Close, Ashton, Cable, Doran, & MacLaren, 2004). If this were the case, exposure to cryotherapy may have actually slowed removal of these by-products from the muscle tissue after exercise. Alternatively, the duration of the exercise bout may have been insufficient to elicit significant lasting soreness in either group, as algometer measures did not differ from baseline in either group. In the study by White et al. (2014) eight participants completed the exercise protocol a total of 4 times in a repeated measures crossover design. It is known that repeated exposure to a muscle-damaging stimulus results in a reduction in the symptoms observed following the initial insult. This is referred to as the repeated bout effect (McHugh, Connolly, Eston, & Gleim, 1999; McHugh, 2003) and work by Nosaka, Sakamoto, Newton and Sacco (2001) suggests that the protective effect for most criterion markers of strenuous exercise (including muscle soreness) lasts at least 6 months after the initial bout. As there was only 1 week separating each of the four trials, it is likely that the repeated bout effect would have influenced some of the outcome measures reported in the study. Whilst soreness is

arguably the most commonly reported subjective marker, other perceptual measures can be used to gauge the effectiveness of cryotherapy as recovery intervention.

2.5.2.2. Other perceptual measures

There are very few studies that utilise a holistic approach to recovery but Broatch et al. (2014) also recorded self-assessments of readiness for exercise, fatigue, vigour, sleepiness, pain, and belief of recovery effectiveness. The results showed that CWI participants demonstrated an increased perception of physical readiness and vigour following their recovery intervention than those in the control group. A further study by Al Haddad et al. (2012) investigated the effects of CWI on heart rate variability and subjective ratings of wellbeing in highly trained swimmers. Over the course of a week, participants continued with their normal training load (5 days a week for approximately 21 hours a week) and were exposed to either 5 min of seated recovery or 5 min of CWI at 15°C. The results showed that participants in the CWI group reported higher sleep quality on days 2, 3 and 4 compared to the control group. The study did not investigate any blood or performance markers, but the results suggest that CWI can have a beneficial impact on subjective markers of wellbeing and recovery. These studies lend weight to the suggestion that many of the benefits associated with cryotherapy exposure, may more accurately be attributed to a placebo effect.

As already mentioned, adequate sleep is an important element of recovery following training (Halson, 2014b; Halson, 2013). However, despite this, there appears to be a scarcity of literature examining the influence of cryotherapy on sleep patterns following strenuous exercise. In contrast to the findings from the Al Haddad et al. (2012) study mentioned previously, a more recent study from Pesenti, da Silva, da Silva, Frisseli and Macedo (2018) reported no difference between CWI and a control

intervention on perceptions of sleep quality following a quadriceps fatiguing protocol. The contrasting findings may be due to the use of a repeated, versus a single exposure protocol. As such, there is scope to further examine the impact of repeated cryotherapy exposure on sleep quality in trained individuals.

Recent systematic reviews and meta-analyses on both CWI (Garcia, Ribeiro, Mota, Hida, & Júnior, 2016; Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015; Machado et al., 2016; Peake, 2017; Tipton, Collier, Massey, Corbett, & Harper, 2017) and WBC (Bleakley et al., 2014) suggest that cryotherapy can offer some benefits compared to control interventions in the management of muscle soreness and perceptions of recovery post exercise, but there is little evidence of any functional benefits, as will be discussed in the next section.

2.5.2.3. Functional and performance measures

There is conflicting evidence relating to the influence of cryotherapy on performance markers following strenuous exercise. Jakeman, Macrae and Eston (2009) reported that a 10 min CWI exposure at 10°C had no beneficial effects on recovery (assessed via MVC of the quadriceps) compared to a control group following 100 maximal CMJs. These results were echoed by Ascensão et al. (2011) who used the same CWI application and reported no differences in sprint time or CMJ height compared to a control group following completion of a 90 min soccer match. Findings from these studies may be explained at least in part by the outcome measures utilised in the study; the performance variables reported are not specific to the exercise stress utilised. For example, the findings from the Jakeman et al. (2009) study may have differed if they had used CMJs as a performance measure, and the use of a controlled soccer specific test such as the LIST may have elicited different results in the Ascensão et al. (2011) study. Furthermore, Broatch et al. (2014) reported that 15 min of CWI at 10.3 ± 0.2°C was not sufficient to attenuate

strength decrements following 4 x 30 sec maximal sprints on a cycle ergometer. However, one of the main mechanisms associated with cryotherapy is the capacity to minimise swelling and soreness resulting from structural muscle fibre damage. The ineffectual results reported in the Broatch et al. (2014) study may be due to the high intensity cycling protocol which would likely elicit predominantly metabolic damage. Conversely, Ingram, Dawson, Goodman, Wallman and Beilby (2009) reported that 2 x 5 min CWI exposures separated by 2.5 min was sufficient to attenuate decrements in sprint performance compared to a control group following an 80 min simulated team sport protocol. Barber, John, Brown and Hill (2017) implemented the same CWI protocol to examine functional recovery following a simulated rugby union protocol and reported that muscle function, measured via CMJ and MVIC of the knee extensors, was improved compared to a control group at 24 and 48 hours post exercise.

A study from Fonda and Sarabon (2013) investigated the influence of WBC on recovery following hamstring-damaging exercise. They reported that knee flexion rate of torque development recovered significantly faster in the WBC group compared to the control condition. However, WBC did not positively alter any other functional markers of recovery, so the authors were hesitant to recommend WBC as a recovery intervention post exercise. These results are supported by a later study from Ferreira-Junior et al. (2015) who reported that a single session of PBC was sufficient to attenuate decrements in peak knee extensor torque at 72 and 96 hours post completion of 5 x 20 drop jumps compared to a control group.

Some of the literature reporting positive influences of cryotherapy on performance measures has utilised repeated bouts over a number of consecutive days. Montgomery et al. (2008) investigated the effects of daily CWI exposure on basketball specific outcome measures during a competitive 3 day basketball tournament. The authors reported that participants in the CWI group demonstrated

smaller vertical jump performance decrements across the tournament than the control group. Similarly, their results showed that CWI was beneficial in maintaining 20 metre sprint time compared to the control group (small 0.02 sec vs moderate 0.04 sec increase respectively). Similarly, Ziemann et al. (2012) demonstrated that a 5 day course of WBC treatment allowed professional tennis players to maintain a higher level of stroke efficiency than participants in a control group during a competitive tennis tournament. The authors postulated that cryotherapy treatment may delay or diminish mental deterioration and fatigue. This theory is supported by Rowsell, Coutts, Reaburn and Hill-Haas (2009) who stated that participants exposed to CWI once a day during a 4 day simulated soccer tournament reported lower perceptions of general fatigue compared to a control group. A meta-analysis from Leeder et al. (2012) suggests that CWI appears to be effective in improving recovery of muscle power, but that there was little evidence to suggest that CWI can accelerate recovery of muscle strength. Similarly a literature review from Rose et al. (2017) suggests that applied studies demonstrate evidence of a positive dose-response relationship between WBC post exercise and the recovery of soreness, muscle function, and markers of inflammation.

The findings from these studies indicate that whilst a single cryotherapy exposure is likely to have little impact on functional recovery that there may be some benefit when used on consecutive days during a tournament scenario. However, it is possible that habitual use may impact on longer term adaptations to training and this is discussed in section 2.5.5.

2.5.2.4. Intramuscular proteins

The presence of CK, myoglobin (Mb) or lactate dehydrogenase (LDH) in the plasma is purported to indicate muscle membrane disruption following exercise (Clarkson & Hubal 2002; Clarkson & Sayers 1999). Roberts et al. (2014), Ascensão et al. (2011)

and Bailey et al. (2007) all reported decreased levels of circulating Mb in the experimental group compared to the control group up to 5 hours post CWI intervention (10 min at 10°C in all cases), however, from 24 h onwards there were no differences in Mb between groups. It is possible that blood flow, and ultimately levels of circulating proteins, was attenuated in the cryotherapy groups for the first few hours post exercise, which could explain the initial difference between groups. Roberts et al. (2014) did not report any CK data, but the remaining 2 studies differed in their findings; Ascensão et al. (2011) reported decreased levels of CK at 24 and 48 hours following CWI compared to the control group but Bailey et al. (2007) reported no significant difference in levels of CK compared to the control group at any time point despite a trend for CWI to have lower values. It is worth noting that absolute CK values for the experimental group were approximately 50% higher (≈ 1200 U/L vs ≈ 800 U/L) in the Bailey et al. (2007) study compared to the Ascensão et al. (2011) study at 24 h post exercise. Participants in the Ascensão et al. (2011) study were trained soccer players, whilst participants in the Bailey et al. (2007) study were classed only as 'healthy'. As such, participants in the latter study may have experienced a far greater magnitude of structural damage, and resultant secondary damage, which could not be successfully attenuated with CWI exposure.

There are also conflicting results relating to the effect of WBC on intramuscular proteins following exercise. Banfi et al. (2009) reported that 5 x 2 min bouts at -110°C over 5 days reduced CK compared to baseline levels in national level rugby players during continued training. However, Hausswirth et al. (2011) reported that 3 x 3 min bouts at -110°C over 3 days did not affect CK values compared to a passive recovery group following a 48 min simulated trail run. The participants in both studies were highly trained athletes but observed differences could be attributed to differences in study design. The Hausswirth et al. (2011) study used a small sample size (*n*=9) and all participants completed the exercise protocol a total of 3 times in

non-adjoining weeks. As already discussed, the repeated bout effect could have influenced the magnitude of muscle damage experienced by participants in the second and third trial, confounding the final results. Also, it is worth noting that neither study employed a control group but instead took baseline values for all DVs after a similar week of training. Although they stated that training volume did not differ between the baseline and experimental treatments, this was either not quantified, or only monitored via heart rate data which may not adequately reflect training load (Buchheit, 2014). As such, these results should be interpreted with caution.

These studies suggest that cryotherapy may attenuate increases in intramuscular proteins post exercise, although it is possible this may be due to alterations in limb blood flow after cold exposure. However, the body of evidence remains equivocal and further studies utilising robust methodologies are required to further knowledge in this area.

2.5.2.5. Inflammation

As already discussed, the potential of cryotherapy to affect inflammation after strenuous exercise has been proposed as one of the key underpinnings of its reported success. In order to investigate alterations in circulating IL-6 post exercise Pournot et al. (2011) utilised repeated WBC exposures compared to a passive control following a simulated 48 min trail run, and Broatch et al. (2014) compared different water immersion protocols (CWI vs thermoneutral vs thermoneutral placebo) following 4 x 30 second sprints on a cycle ergometer. Findings from both studies demonstrated that despite significant time effects in all groups, the implementation of a cryotherapy intervention did not influence the magnitude of circulating IL-6 compared to the other conditions. However these results are in contrast to those reported by Roberts et al. (2015) who reported that IL-6 was

elevated in the CWI group 1.5 and 2 h post exercise compared to an active recovery control, suggesting that cryotherapy has the potential to increase and/or prolong the acute inflammatory response.

These conflicting results may be explained by differences in the timing of data collection post exercise; the Roberts et al. (2015) study was the only one that examined values between 1 and 24 h post and research has demonstrated that IL-6 peaks immediately after exercise and may return to baseline as soon as 4 hours post exercise (Ostrowski et al., 1999). Therefore, where studies aim to assess the efficacy of any intervention on circulating IL-6, blood samples should be taken not only post-exercise, but also in the first few hours post intervention. The results from Roberts et al. (2015) suggest that cryotherapy may only affect IL-6 activity for a short period of time, possibly as a result of altered blood flow parameters. It is therefore possible that these changes are negated when tissue temperatures return to baseline values, although without blood flow or intramuscular temperature data from any of the studies, this is only speculative.

Alternatively, the difference in results may be explained by the use of different exercise protocols. Roberts et al. (2015) utilised a high intensity resistance training session, whereas the other protocols utilised endurance running (Pournot et al., 2011) and short duration sprint cycling (Broatch et al., 2014) respectively. Despite Broatch et al. (2014); Pournot et al. (2011) and Roberts et al. (2015) using recreationally active or trained participants, the exercise protocol implemented by Roberts et al. (2015) was a high volume, heavy load maximal resistance training session which may have induced greater structural damage than the other two exercise bouts.

CRP is mediated by the secretion of IL-6 and as such, is another useful marker to examine inflammation following exercise (Stewart et al., 2007). In support of the

findings from Broatch et al. (2014) who reported no influence of cryotherapy on IL-6, Ingram et al. (2009) reported that 2 x 5 min CWI exposure at 10°C following an 80 min simulated team sport exercise did not influence values of CRP compared to a control group who received no treatment. In contrast, Pournot et al. (2011) reported that a 3 min WBC exposure at -110°C was sufficient to attenuate CRP compared to a passive recovery group (123 versus 515% increase respectively) 24 h following a 48 min simulated trail run, despite no between group differences in IL-6. The reported increase in CRP for the control group 24 h post exercise was comparable between studies (6-fold). The contrasting findings may be explained by the different cryotherapy applications utilised within the studies (CWI versus WBC), although the responsible mechanism(s) remain unclear.

All the studies mentioned in the previous paragraphs assessed the impact of cryotherapy on inflammation by analysing blood samples. However, as already discussed, cold application can influence peripheral blood flow which could in turn influence the extent to which inflammatory mediators are evident.

A recent study from Peake et al. (2017) utilised biopsies to examine the influence of CWI or active recovery on exercise induced expression of several pro- inflammatory cytokine and chemokine genes in muscle tissue. Their results demonstrated that CWI exposure following resistance exercise did not alter the inflammatory response (assessed via IL-1β, TNF-α, IL-6, monocyte chemoattractant protein 1, chemokine (C-C motif) ligand 4, chemokine (C-X-C motif) ligand 2 and IL-8) compared to active recovery. This study was the first to assess inflammation within the muscle, and the findings not only contradicted the authors' hypothesis, but also a large proportion of the previous literature suggesting that CWI may restrict inflammation and cellular stress responses in muscle following exercise. Additionally, by employing an active recovery condition rather than passive rest, the findings are more likely to be reflective of typical athletic practice. The authors also stated that previous research

shows little or no difference in circulating cytokines between active and inactive/sedentary recovery after exercise (Andersson et al., 2010).

Currently, research examining whether cryotherapy can attenuate inflammation following strenuous exercise is equivocal. Where positive results have been reported it is possible that by limiting the inflammatory response, cryotherapy treatment may attenuate associated increases in soreness and decrements in muscle strength and power which can impact negatively on subsequent performance. Furthermore, there is some evidence to suggest that CWI can exacerbate the inflammatory response post exercise, contradicting previous literature reporting positive effects.

2.5.3. Direct comparison of WBC and CWI

Despite a growing body of literature, there are still relatively few papers that directly compare WBC and CWI. Direct comparison studies would afford researchers better understanding of the circumstances under which either treatment is, or is not effective, and would also help to clarify whether one intervention can offer any substantial benefit over the other.

During CWI there is an element of hydrostatic pressure which is not present during WBC treatments. However, a recent study by Leeder et al. (2015) reported that increasing hydrostatic pressure by manipulating body position (seated vs standing) did not provide an additional recovery benefit following high intensity intermittent sprint exercise, and that neither condition offered any recovery benefit compared to a non-immersion control.

Given that many of the reported physiological changes are thought to be temperature dependent, methodological aspects affecting tissue temperature are particularly pertinent (White & Wells, 2013). Despite cryotherapy chambers reaching far lower temperatures than ice baths (≈ -110°C compared to ≈8°C), water is

actually much more efficient at removing heat from the body due to an increased heat transfer coefficient (Wakabayashi, Kaneda, Sato, Tochihara, & Nomura, 2008). Exposure to cryotherapy can be painful at first, and anecdotal evidence suggests that participants tend to find CWI more uncomfortable than WBC, however, a paper by Costello, Culligan, Selfe and Donnelly (2012) found the opposite to be true, although they did not offer an explanation for their findings. Differences in thermal sensation and comfort may be linked to the increased heat transfer coefficient of water compared to air. Although neither water nor air is as efficient as ice at extracting heat from the body, a distinct advantage is that unlike ice, they both facilitate large surface areas of the body to be cooled simultaneously. The literature states that cryotherapy applications which are of a longer duration, are applied to a greater surface area or present a greater thermal gradient are positively correlated with an increased magnitude of tissue temperature change (Janwantanakul, 2009; Machado et al., 2016).

In a study from Costello, Culligan, et al. (2012), the authors investigated differences in muscle, skin and core temperature following either an 8° CWI or -110°C WBC protocol. Their results demonstrated that muscle and core temperatures were decreased by a comparable amount, regardless of the cryotherapy modality; however, WBC elicited greater reductions in skin temperature. The authors acknowledged that due to equipment limitations, superficial muscle temperature was only recorded 60 min post exposure, meaning that greater temperature reductions may have been evident from 0-60 min post intervention. However, as already mentioned, as intramuscular temperatures may remain below baseline levels for a number of hours following exposure, there are implications for oxygen delivery to the muscle post-exercise.

Following on from this, Costello et al. (2014) published a paper comparing 4 min of WBC or CWI (-110°C and 8°C respectively) on knee skin temperature. Their results

showed that skin temperature was significantly reduced from baseline up to 60 min post exposure following both cooling modalities. Despite average and minimum skin temperatures being lower immediately after WBC compared to CWI (19.0 ± 0.9°C vs 20.5 ± 0.6°C average), from 10 to 60 min post, the average, minimum and maximum skin temperatures were lower ($p < 0.05$) following CWI. The authors also noted that neither protocol achieved a skin temperature below 13°C, which is the generally agreed temperature required for an analgesic effect (Bleakley & Hopkins, 2010). As already discussed, the effective cooling of skin and muscle tissue is vital for the success of any cryotherapy intervention. The results reported by Costello et al. (2014) suggest that a single 4 min WBC or CWI exposure may not be sufficient to induce optimal temperature change required to elicit therapeutic effects. The CWI protocol implemented in the study has been cited in the literature by a number of authors (Costello et al., 2012a; Costello & Donnelly, 2011; Gregson et al., 2011) but these studies have predominantly focused on skin temperature and blood flow change in rested participants rather than recovery following strenuous exercise. As such, the results may not be reflective of what would be seen in a real world scenario where CWI exposures are frequently 10 minutes or longer.

More recently, Mawhinney and colleagues (2017) examined the effect of ecologically valid cryotherapy protocols (CWI 8°C for 10 min, or WBC -110°C for 2 min) on post exercise lower limb thermoregulatory, femoral artery and cutaneous blood flow responses. Their results showed that following a continuous cycle exercise protocol at 70% $\dot{V}O_2$ max, CWI elicited greater reductions in blood flow and tissue temperature than WBC. These results are supported by another study from Hohenauer and colleagues (2017) who demonstrated that cutaneous vascular conduction, thigh muscle oxygen saturation and lower extremity skin temperature were significantly reduced in the CWI group compared to PBC following a muscle damaging protocol (5 x 20 drop jumps). As already stated, the greater temperature

reductions seen after CWI may be attributed to the increased thermal conductivity of water compared to air. The greater potential for temperature reduction may mean that CWI can offer greater recovery benefits in terms of perceptual responses, inflammation and function.

A recent paper by Abaïdia et al. (2016) compared the effects of CWI (10 min at 10°C) and WBC (3 min at -110°C) on recovery kinetics following eccentric single-leg hamstring exercise comprising 5 sets of 15 repetitions. Their results demonstrated that there was a very likely moderate effect in favour of CWI for recovery of single and double leg CMJ compared to WBC 72 hours post exercise. Further, there was a moderate reduction in perceived soreness and a moderate increase in perception of recovery at 24 and 48 h post respectively. The authors concluded that CWI was more effective than WBC in enhancing recovery kinetics for CMJ 72 hours after exercise. It is important to note that the exercise bout employed in the study lacked ecological validity, and that there was no placebo or control group. Therefore, there remains scope to examine these recovery modalities using sport specific exercise stressors and a more rigorous placebo-controlled study design. As a result, the findings should be interpreted and implemented cautiously. A similar study from Hohenauer et al. (2017) utilised a drop jump protocol (5 x 20) to investigate the influence of CWI (10°C for 10 min) or PBC (-60°C for 30 sec, −135°C for 2 min) on recovery. The authors reported no difference in muscle soreness between groups, and also stated that muscle function, assessed via MVC and CMJ, had returned to baseline values after 24 h in both groups. The fact that muscle function variables had returned to baseline by 24 h may indicate that the muscle damaging protocol utilised in the study was insufficient to elicit significant mechanical damage. All participants in the study were moderately trained endurance athletes, and as such repeated drop jumps may not have represented a ‘novel’ exercise stress in terms of SSC activity.

Holmes and Willoughby (2016) conducted a review in order to try and understand the effectiveness of WBC compared to CWI and the implications for recovery after exercise. The authors concluded that, despite its growing popularity, many of the alleged benefits of WBC were based on anecdotal evidence. Further, the authors stated that much of the WBC research was observational and did not utilise a control group, and that randomised, controlled studies were needed to better understand any potential benefits. In addition, further research is warranted to investigate the effectiveness of CWI versus WBC following different applied exercise stresses.

At the present time, there is very little literature directly comparing WBC and CWI. The available evidence is predominantly concerned with the capacity of different cryotherapy applications to affect a decrease in skin and or muscle temperature, rather than the potential to enhance recovery following strenuous exercise. There is currently a paucity of literature investigating differences in blood markers, perceptual or performance measures following WBC and CWI. Whilst the literature relating to changes in temperature and blood flow can elucidate underpinning mechanisms related to cryotherapy application, the impact on functional and performance markers is crucial for athletes and practitioners. Further research in this area would help inform appropriate interventions to maximise or accelerate recovery and ultimately improve performance. Additionally, there are a lack of studies utilising adequate placebo-controlled deigns, and there is evidence to suggest that some of the reported benefits of cryotherapy may be related to the placebo effect (Broatch et al., 2014; McClung & Collins, 2007), and this will be discussed in the following section.

2.5.4. Importance of the placebo effect

An increasingly important facet of recovery research is understanding the importance and impact of placebo interventions and the contribution of expectance effects. Whilst placebos and expectancy are not the same thing, a causal effect has been investigated and assumed (Kirsch, 1985). The placebo effect can be described as any effect attributable to a pill, potion or procedure, but not directly related to its pharmacologic or other properties, whereas expectancy relates to an individual's belief regarding the efficacy of an administered substance or treatment (McClung & Collins, 2007). It follows that expectance effects arguably play a considerable role in any reported benefits of an inert placebo or other sham intervention. The importance of recognising the potential impact of the placebo effect and/or expectance effect in cryotherapy research is particularly significant as effective blinding is incredibly difficult to achieve (Higgins et al., 2017; Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015). A large proportion of previous cryotherapy research has been conducted utilising control, rather than placebo groups, which may have resulted in a falsely inflated sense of the efficacy of various cryotherapy interventions. A final confounding issue is that studies conducted using control groups cannot easily be compared to other investigations utilising placebo or alternative intervention strategies. Another way to try and account for expectance effects is to use effective placebo interventions for those participants not in the treatment condition.

One study that successfully implemented a placebo intervention alongside a CWI protocol came from Broatch et al. (2014). Within the study, participants were assigned to one of 3 conditions; CWI, thermoneutral water immersion (control) or a placebo thermoneutral water immersion. For the placebo condition, a deidentified, pH-balanced, dissolvable skin cleanser was added to the water in sight of participants. Findings from the study revealed that the placebo intervention was as

effective as CWI for the recovery of muscle strength 48 h after completion of a high intensity interval training session, and that both CWI and the placebo were superior to the control condition. After reading the study information provided to them, participants in the placebo condition rated the expected benefits of their assigned recovery protocol more highly than the ratings of participants in control condition. Furthermore, participants in the placebo group reported greater mental readiness for exercise and lower pain levels immediately post recovery compared to the control group.

These findings indicate that the hypothesised physiological and perceptual benefits of CWI are likely attributable, at least in part, to a placebo effect. Therefore, future cryotherapy research should utilise placebo-controlled study designs in order to minimise any potential influence of this confounding factor.

2.5.5. Influence of cryotherapy on training adaptations

Broadly, recovery interventions may work in one of two ways after exercise; by exploiting positive changes (e.g. increased muscle protein synthesis stimulated by resistance exercise) or by suppressing seemingly negative changes (e.g. increased inflammation as a result of structural damage). Where recovery interventions promote or enhance the expected process after exercise, there is unlikely to be a maladaptive response after repeated use. However, cryotherapy interventions fall into the latter category, and the suppression of a natural physiological response, such as acute inflammation, could inhibit longer term training adaptations (Heaton et al., 2017; Higgins et al., 2017; Roberts, Raastad, et al., 2015). Cryotherapy has been lauded specifically for its potential to reduce the inflammatory response by reducing the stimulus inciting the activation of pathways associated with secondary damage (White & Wells, 2013). By reducing inflammation and resultant secondary damage initiated by cellular damage to muscle fibres, the magnitude of repair required to achieve pre-exercise structural and functional integrity is also reduced,

ultimately leading to a shorter recovery time (White & Wells, 2013). However, whilst inflammation and associated pain could be categorised as undesirable symptoms of skeletal muscle damage post exercise, the inflammatory response is a key element of the process preceding the regenerative phase of muscle damage leading to adaptation and the remodelling of muscle tissue (please refer to figure 2.1).

In terms of the influence of cryotherapy on adaptations to training, a number of studies have been conducted using both endurance and resistance based exercise stresses. However, the subsequent section will focus solely on the literature relating to resistance exercise as this is pertinent to the thesis. Similarly, the following section will focus on CWI as this is most relevant to the current investigation, and at the time of writing, there does not appear to be any data relating to habitual WBC exposure and training adaptations. Fröhlich et al. (2014) and Roberts et al. (2015) investigated the effect of repeated CWI on adaptations to strength training. In the study by Fröhlich et al. (2014) participants were tested for 1 and 12 repetition maximum (RM) of a single leg curl before and after a 5 week strength training programme. Immediately after each training session, one limb was cooled for 4 x 3 min at ~12°C whilst the control limb was not cooled. After completion of the training programme the authors reported significant increases for both 1 and 12RM, with a tendency for a ‘large’ intervention effect with higher values for the control limb. The authors stated that whilst the effects were small, long term strength adaptations appeared to be negatively impacted by CWI.

In the study by Roberts et al. (2015) twenty-one physically active male participants completed progressive strength training twice a week for 12 weeks. Those in the experimental group completed a 10 min CWI exposure, and those in the control group completed 10 min of active recovery following every exercise session (≈ 24 sessions in total). At the conclusion of the 12 week programme, the authors reported that participants in the CWI group exhibited smaller increases in strength

and muscle mass compared to the control group. Moreover, participants in the CWI group demonstrated no change in total isokinetic work, type II muscle fibre cross sectional area or number of myonuclei per fibre, despite an increase in all measures in the control group. The authors attributed these findings to the fact that CWI blunted the activation of key proteins and satellite cells implicated in the process of hypertrophy for up to 2 days after strength exercise, evidenced in a follow up study utilising biopsy data (Figueiredo, Roberts, Markworth, & Barnett, 2016). As part of the second study, participants performed 2 separate bouts of high intensity resistance exercise followed by active recovery or CWI. Muscle biopsy samples were taken before resistance exercise, and at 2, 24, and 48 h post exercise, in order to assess the impact of the recovery interventions on ribosome biogenesis. The findings demonstrated although resistance exercise increased p38 MAPK (a stress-activated kinase) the effect was markedly blunted following CWI. The authors stated that this had downstream effects on many factors involved in ribosome biogenesis, including MNK1, eIF4E, UBF, cyclin D1, and c-Myc protein.

Therefore, habitual CWI use may result in a downregulation of muscle protein regeneration and satellite cell activation, which could negatively impact on performance where strength or power characteristics are fundamental to success (Fuchs et al., 2018). A possible explanation for this phenomenon is the result of a reduced inflammatory response, leading to reduced satellite cell stimulus and proliferation. However, as previously discussed, Peake et al. (2017) demonstrated that CWI did not attenuate the inflammatory response post exercise compared to active recovery, therefore cold induced vasoconstriction may not be responsible for the restriction of kinases involved in the mTOR pathway.

Peake et al. (2017) states that whilst changes in tissue temperature, blood flow and perceptions of soreness post CWI are well reported and documented, many of the secondary aspects remain more speculative. In a recent review, Broatch et al. (2018)

examined the available literature relating to the impact of CWI on adaptations following resistance training. The authors concluded that the current body of evidence indicates either no effect, or a negative effect of CWI on the molecular mechanisms regulating key resistance training adaptations such as increased strength and muscle mass. Where negative effects have been reported, this may be as a result of inhibited mTOR signalling. The authors also state that whilst the impact of repeated CWI exposure on muscle mass has been examined, there is currently no literature relating to changes in muscle pennation angle which may also contribute to increases in strength.

To date, research supporting or refuting the use of post-exercise CWI following resistance exercise remains scarce (Broatch et al., 2018). Further research is warranted to understand the impact of habitual CWI exposure on human skeletal muscle adaptations during real-world training scenarios. Moreover, the inclusion of additional measures such as ultrasound and electromyography may help to elucidate responsible mechanisms.

2.6. Summary

It is has been extensively documented that strenuous exercise can result in a number of undesirable symptoms such as disturbances in perceptual markers, decreases in force generating capacity, and inflammation (Armstrong, 1984; Bobbert, Hollander, & Huijing, 1986; Byrne et al., 2004; Chatzinikolaou et al., 2010; Cheung et al., 2003; Clarkson & Hubal, 2002; Cleak & Eston, 1992; Finsterer, 2012; Gulick & Kimura, 1996; Proske & Morgan, 2001; Pyne, 1993; Raastad et al., 2010; Takekura, Fujinami, Nishizawa, Ogasawara, & Kasuga, 2001; Tidball, 2005). The magnitude of change and time course of recovery of these symptoms differs depending on the mode and duration of exercise performed. Whilst prolonged endurance exercise leads to greater disturbances in inflammatory markers,

resistance exercise likely results in greater disturbances in isometric and isokinetic strength. Therefore there is a need to evaluate the effectiveness of any recovery intervention following different exercise modes.

Despite a growing body of literature focusing on cryotherapy and its potential to limit structural muscle damage, inflammation and perceptual responses following exercise and promote subsequent recovery, there are still considerable gaps in the research. There are many papers relating to the use of cryotherapy as a recovery intervention, but differing exercise stresses, methods and modes of application and outcome measures means it is difficult to draw comparison between results. Minett and Costello (2015) highlight the fact that whilst emphasis is placed on the specificity of exercise prescription and training, the same principle is rarely applied to post-exercise recovery strategies. It cannot simply be assumed that any one recovery strategy will achieve universal success; recovery is not a ‘one size fits all' concept. Currently, there is a need to establish whether CWI or WBC can have an impact following different types of exercise stress (endurance or resistance), and also whether WBC can offer any additional benefits than more traditional strategies like CWI.

As already mentioned, there are only a handful of studies directly comparing WBC and CWI and currently there is little data looking at performance or perceptual markers. Very few, if any studies have taken a holistic approach to investigating recovery, instead focusing on blood markers (damage, inflammation or oxidative stress) *or* performance measures *or* perceptual markers.

Moreover, there is little data examining the effect of cryotherapy over a resistance training block. Currently, it does not appear that a placebo-controlled study design has been used to investigate adaptations to resistance training, and the inclusion of PINP and PIIINP could offer mechanistic insight. Furthermore, there are no studies

using an ecologically valid exercise stress and a bilateral cooling protocol that have examined whether CWI influences adaptation over a period of less than 12 weeks. As already discussed it is possible that repeated cryotherapy exposure over a prolonged period of time may result in maladaptive responses to training which could negatively impact on future performance (Peake, 2017). Therefore, understanding not only acute responses to cryotherapy but also potential (mal)adaptations following habitual exposure is pertinent to athletes and coaches employing such interventions.

Finally, in all the studies presented as part of this thesis, participants who were not allocated to a cryotherapy intervention group were given an alternative (sham) intervention appropriate to the exercise mode utilised, in an attempt to minimise confounding placebo effects. Placebo interventions were determined based on nutritional supplements that have strong scientific evidence relating to their capacity to enhance recovery and/or performance. It was hoped that by implementing placebo interventions that the results from the studies would be more robust and with greater ecological validity, than studies using control groups. Given the increasing focus on recovery and ease of access to a wide range of recovery modalities, it is unlikely that any competitive athlete would neglect to implement any form of recovery strategy at all.

Chapter 3

General Methods

3.1. Overview

This thesis comprises 3 progressive studies investigating the influence of cryotherapy interventions on markers of recovery and adaptations to training following exercise. The methods described in this chapter are those that are common to at least 2 of the studies. All investigations were conducted in the Sport Science labs, or within the grounds of Allianz Park, at Middlesex University following receipt of institutional ethical approval (Appendix 1). For all studies, healthy, active males were recruited. Participants were excluded from taking part in the studies if they had any injuries or illnesses that prevented them from completing any of the prescribed exercises or tests. In addition, participants with Raynaud's disease, circulatory problems or needle phobia were excluded from the study. For study 1, participants were endurance trained runners capable of completing a marathon distance within 4 and a half hours. For studies 2 and 3 participants were required to have a minimum of 12 months experience of resistance training and be capable of safely completing high load lower body resistance exercises. Participants were recruited through targeted social media and email communication. After volunteering, participants were provided with both verbal and written information about the appropriate study including possible risks and benefits (Appendix 2), before providing informed consent (Appendix 3) and completing a comprehensive health questionnaire (Appendix 4). Participants were familiarised with testing procedures before the commencement of each study. For each study, participants were asked to maintain their habitual diet and avoid any nutritional supplements, anti-inflammatory drugs or alternative recovery interventions for one week before, and up until completion of the study. Participants were asked to arrive at the laboratory in a rested state, having avoided strenuous activity for a minimum of 48 hours. As far as possible, testing sessions for each participant were conducted at the same time of day to minimise the potential influence of circadian rhythm.

3.2. Experimental design

All the studies utilised an independent measures design. For study 1, participants were allocated into groups using simple randomisation, whereas for studies 2 and 3, participants were matched into groups based on a ratio of their predicted 1RM for the back squat and their lean body mass (stratified randomisation). Lean mass was assessed via dual x-ray absorptiometry (DXA) during the familiarisation sessions (Roberts et al., 2015). Recruitment for all three studies continued throughout the data collection phases, but interventions were counterbalanced across testing days (study 1 and 2) or testing blocks (study 3) to minimise further confounding effects. For study 1, participants were familiarised with all testing procedures on the same day as baseline testing, and then returned to the lab to complete post testing at 24 and 48 hours. For studies 2 and 3, participants were familiarised with testing procedures and completed assessment of their RMs at least 48 hours (study 2) or 1 week (study 3) before the baseline session. For study 2 participants returned to repeat DV's at 24, 48 and 72 hours, and for study 3, participants repeated DV's after 4 weeks (mid-point) and 8 weeks (post testing) of training.

3.3. Dependent variables

3.3.1. Dual x-ray absorptiometry (DXA) scan

Body composition of all subjects in studies 2 and 3 was assessed using a DXA scanner (fan beam, Lunar Prodigy 4, GE Medical Systems, Lunar, Madison, WI, USA). Participants were required to attend the lab in a euhydrated state to ensure accurate measurements (Horber, Thomi, Casez, Fonteille, & Jaeger, 1992). Participants were required to lie on their back on the scanner and remain still for the duration of the assessment (6-11 minutes). Participants were asked to wear minimal clothing (shorts and/or underwear) and to remove any jewellery or items with metal zips or fastenings. Test–retest reliability of DXA anthropometric measures have

previously been derived from repeated scans (Bilsborough et al., 2014). DXA demonstrated excellent precision for fat-free soft tissue mass (CV = 0.3%) and acceptable reliability for fat measures (CV: fat mass = 2.5%, percent body fat = 2.5%).

3.3.2. Perceived soreness

For studies 1 and 2, participants were asked to indicate perceived levels of muscle soreness of the lower limbs using a VAS (Appendix 5) at each testing session. For study 3, participants completed this scale online at the appropriate time points, as described in the relevant chapter. The scale was numbered, where 0 indicated no soreness on movement, and 10 indicated that the muscles were too sore to move. Participants were asked to rate their perceived level of soreness during a squatting movement. With hands on hips and feet shoulder width apart, participants were asked to squat to a knee joint angle of approximately 90º and then rate the respective level of perceived soreness for the lower limbs on the scale. This method has been used successfully in previous studies to monitor changes in perceptions of pain following exercise (Vaile, Halson, Gill, & Dawson, 2008; Vaile, Gill, & Blazevich, 2007).

3.3.3. Daily analysis of the lifestyle demands of athletes (DALDA)

For study 1 and 2, participants completed a DALDA questionnaire (Appendix 6) on each testing day. For study 3, participants completed the questionnaire online and the time course of completion is described in the relevant chapter. The questionnaire comprises 2 sections; part A identifies potential sources of stress (including home life, work, sleep and sports training), whilst part B allows individuals to rate stress reactions symptoms as worse than normal, normal, or better than normal. Several studies have successfully utilised the DALDA questionnaire to monitor fatigue and recovery (Hogarth, Burkett, & McKean, 2015; Robson-Ansley,

Gleeson, & Ansley, 2009). Only results for part 'B' are reported in each study, after Halson et al. (2002) and Coutts et al. (2007) stated that there were only significant changes in the second part of the questionnaire following a period of intensified training.

3.3.4. Calculation of repetition maximums (RMs)

The calculation of RMs was completed during the familiarisation session for study 2 and 3, based on the protocol of the National Strength and Conditioning Association (Baechle, Earle, & Wathen, 2000). For study 2, 4RM was determined for each exercise, and then predicted 1RM for the back squat was calculated using the Wathen prediction equation (Wathen, 1994). For study 3, 4RM was determined for the ¼ squat and split squat and 6RM was determined for hip thrusts and Romanian deadlifts, in order to provide the training loads for the first session of the strength training block.

After a thorough warm up, participants were asked to select a load that would easily allow 6-10 repetitions. Following a 2-minute rest, an additional 20% load was added and participants completed a further 6–8 repetitions. This process was repeated (increasing the load by 5 – 10% each time) until the participant's 4 or 6RM was determined. Loss of technique during the exercise was deemed as an unsuccessful lift. If a miscalculation was made whereby the participant was unable to complete the required number of repetitions, they were provided with up to 15 minutes rest, before attempting the set again, with a reduced load.

3.3.5. Peak torque and maximal voluntary isometric contractions

Peak knee extensor torque and MVIC were measured using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). Following a standardised warm-up, participants were seated on the dynamometer with their upper body secured in place to prevent any extraneous movement. The involved

limb was fixed to the input arm of the dynamometer ensuring correct alignment of the rotational axis of the dynamometer and the lateral femoral epicondyle to ensure only unilateral movement about the knee joint was possible. The range of motion for each participant was manually determined by the investigator.

For studies 1 and 2 MVIC of the knee extensors was assessed at 90° (Costello, Algar, et al., 2012; Hill et al., 2014), and for study 3 it was additionally measured at 30° to investigate the angle-torque relationship (Narici et al., 1996). Participants completed 2 warm up trials at ~ 50% and ~75% of maximum, before completing 3 maximal 5 second efforts for each angle. Participants were instructed to contract as fast and as hard as possible and the highest value was taken for analysis.

Peak torque measurements were recorded at $60°\cdot s^{-1}$ (studies 1, 2 and 3) and $180°\cdot s^{-1}$ (study 3). Following 2 warm up trials participants performed 3 maximal efforts by forcefully extending and flexing the knee against the dynamometer arm. The highest concentric torque value achieved for the quadriceps was taken and used for analysis. Intra-class correlation coefficients for knee extension peak torque at $60°\cdot s^{-1}$ and $180°\cdot s^{-1}$ have been reported as r = 0.95 and r = 0.96 respectively (Feiring, Ellenbecker, & Derscheid, 1990). For study 1, values were recorded directly from the System 3 software, but for studies 2 and 3 data were collected using a laptop running Powerlab software, interfaced with the dynamometer. By utilising Powerlab, it was possible to sample data at a much higher rate (2000 Hz versus 100 Hz) in order to gain more accurate peak torque values.

For study 1, peak knee extensor torque and MVIC were measured on the self-reported dominant limb, whereas in an effort to streamline data collection, all measurements were recorded on the right limb for studies 2 and 3. Given that all exercises were performed bilaterally, and that all results were reported as change

over time, it is unlikely that utilising the right limb as opposed to the self-reported dominant limb would have significantly impacted on the results.

3.3.6. Peak force and rate of force development

For studies 2 and 3 participants were asked to perform an isometric squat in order to calculate peak force and RFD. Isometric squat parameters were measured using a portable force platform (Kistler, Switzerland), interfaced with a laptop and placed inside a custom designed rack (Absolute Performance, Cardiff, UK) allowing for adjustable bar height, and a sampling rate of 1000 Hz. For each participant, the bar was set in line with the base of their sternum, in an attempt to ensure that the isometric squat was performed in the mid-range of a back squat movement. The bar position was replicated at each testing session. Participants were asked to maintain a stable position under the bar whilst applying minimal pressure. Participants were asked to drive straight up as fast and as hard as possible against the bar and to maintain the contraction for 3 seconds (Maffiuletti et al., 2016). Three trials were completed at each testing session with a 3 minute rest between efforts. If there was any sign of a visible countermovement, the trial was deemed void and repeated after a 3 minute rest. RFD was calculated from the force–time curve as the slope of the linear function from 50 to 100 (study 3) and 100 to 200 milliseconds (studies 2 and 3). Changes in the early phase of a contraction (<100 ms) can be attributed to fatigue or other neural factors, whilst changes in the later phase (>100 ms) tend to reflect alterations to contractile elements of skeletal muscle (Maffiuletti et al., 2016; Peñailillo et al., 2015). Data were sampled at 1000 Hz and the isometric peak force was determined as the maximal force recorded from each trial minus body mass. Peak isometric force was taken and used for analysis. The peak RFD value from 100-200 ms was used for analysis as well as the corresponding 50-100 ms value from the same trial. Initiation of the contraction was identified as the first force value greater than 5 standard deviations of the baseline period (Chavda et al., 2017).

3.3.7. Reactive strength index (RSI)

Participants were instructed to drop from a platform at a height of 30 cm and then jump vertically for maximum height as quickly as possible. Emphasis was placed on minimum ground contact time, whilst maintaining maximum jump height. Participants were required to keep their hands on their hips for the duration of the movement, and perform 3 maximal jumps at each testing point. Reactive strength index (RSI) for each effort was calculated by dividing flight time by ground contact time (Flanagan & Comyns, 2008) and peak RSI values were used for statistical analysis. For study 1, data was collected using a portable jump mat (Kinematic Measurement System, KMS, Fitness Technology, Australia), and for studies 2 and 3 a portable force plate (Kistler, Switzerland). The force plate was interfaced with a laptop and data were collected at 1000 Hz.

3.3.8. Countermovement jumps (CMJ)

From a relaxed standing position, participants made a countermovement to a squat position (self-selected depth) before jumping vertically for maximal height. Each jump was performed in a continuous movement with hands remaining on hips for the duration. Three jumps were recorded at each testing session. Any efforts that deviated from the prescribed technique were deemed void and repeated. For study 1, data was collected using a portable jump mat (Kinematic Measurement System, KMS, Fitness Technology, Australia), and for study 2 a portable force plate (Kistler, Switzerland) interfaced with a laptop was utilised. Peak jump height values were used for analysis.

3.3.9. Blood sampling

Whole blood samples were collected from the antecubital vein into 4 mL vacutainers for the purpose of assessing structural muscle damage and inflammation. Blood samples were then centrifuged at 3000 rpm for 8 minutes before being aliquoted

and stored at -80°C for later analysis. The timing of blood data collection is described in the relevant study chapters. Blood markers included creatine kinase-M (CK-M), CRP, IL-6 and TNF-α for studies 1 and 2. Blood markers for study 3 are included in the relevant chapter section.

3.3.9.1. CK-M

Plasma CK-M concentrations were measured by simple step enzyme-linked immunosorbent assay (ELISA) (Abcam, Cambridge, UK). The reported assay ranges were 54.3 – 268.9 U/L (study 1), and 0.03 – 2.0 U/L (study 2). The minimum detection concentration (MDC) was 0.01 U/L, and the human serum intra- and inter-assay CV were 3% and 9%, respectively.

3.3.9.2. CRP

Plasma CRP concentration was determined using a quantitative sandwich (QS) ELISA technique (Quantikine, R&D Systems Europe Ltd., Abingdon, UK (study 1), or IBL International GmbH, Hamburg, Germany (study 2)). The MDC for the assays was <1 µg/ml with an intra and inter-assay CV of 6.6 and 8.3% (study 1) and 5.12 and 14.3% (study 2) respectively.

3.3.9.3. IL-6

Plasma IL-6 concentration was determined by a QS-ELISA (Quantikine, R&D Systems Europe Ltd., Abingdon, UK). The reported assay ranges were 3.1 – 300 pg/ml, the MDC was 0.7 pg/ml, and the intra- and inter-assay CVs were 2 and 3.8% respectively.

3.3.9.4. TNF-α

Plasma TNF-α concentration was measured by QS-ELISA (Quantikine, R&D Systems Europe Ltd., Abingdon, UK (study 1) or BioVendor, Brno, Czech Republic

(study 2)). For study 1, the limit of quantification (LOQ), defined as the lowest concentration that could be distinguished from 0 was 0.5 pg/ml. The serum intra- and inter-assay precision, determined by CV were 4.9 and 9.9% respectively. For study 2 the reported assay ranges were 7.8 – 500 pg/ml, the MDC was 2.3 pg/ml, and the intra and inter-assay CVs were 6.0 and 7.4% respectively.

3.4. Interventions

3.4.1. Placebo

Due to the difficulty in successfully blinding participants to cryotherapy interventions, a placebo intervention was utilised in each study. A different placebo intervention was used in each study in an attempt to provide an appropriate alternative (sham) treatment. Details of the placebo interventions are discussed in more detail in the appropriate chapters.

3.4.2. Cold water immersion (CWI)

Within 15 minutes of cessation of exercise participants sat in a mobile ice bath (iSprint Twin, iCool, Cranlea, UK) ensuring their lower limbs and iliac crest were fully immersed. Participants remained in the ice bath filled with water cooled to either 8 degrees (study 1) or 10 degrees (studies 2 and 3) (± 0.5º) for 10 min. The CWI protocol for the first study was based on previous studies from Jakeman, Macrae and Eston (2009) and Bailey et al. (2007) who investigated recovery following eccentric and prolonged intermittent exercise respectively, utilising a single CWI exposure. However, whilst developing the second and third studies, a number of articles were published indicating that immersion temperatures of 10-15°C were used more routinely (Garcia et al., 2016; Hohenauer et al., 2015), and may actually be more beneficial for recovery (Vieira et al., 2016). Increasing immersion temperature by 2°C between study 1 and 2 is unlikely to have a dramatic impact on the findings, but allows for clearer comparison to other literature.

The ice bath was connected to a chiller unit (MiCool, iCool, Cranlea, UK) so that water temperature could be monitored and maintained within the desired parameters for the duration of the treatment. During exposure participants wore shorts and immediately after they were asked to towel themselves dry and change into clean, dry clothing. This protocol is comparable to those utilised in other single exposure studies examining the effects of CWI on various measures of recovery (Ascensão, Leite, Rebelo, Magalhães, & Magalhães, 2011; Roberts, Nosaka, Coombes, & Peake, 2014).

3.4.3. Whole body cryotherapy (WBC)

In studies 1 and 2 the WBC group were exposed to 2 cold treatments in a cryotherapy chamber (CryoClinics, London, UK or CryoAction, London, UK). Participants (up to 2 at a time) spent 3 min in the chamber set to -85°C ± 5°C. Participants then had a 15 min warming period in an ambient room before entering the chamber for a further 4 min bout at -85°C ± 5°C. The protocol was determined in part by the chamber available for the first study; the temperature was controlled electronically rather than with liquid nitrogen. Therefore, the temperature used was the minimum attainable temperature using the electronically controlled chamber. Secondly, WBC procedures cited in the literature normally use a 2.5 – 3 min exposure at -110°C. Due to the warmer minimum operating temperature, we decided to use 2 bouts separated by a 15 min warming period. The protocol was also recommended by the Director of CryoClinics as the standard protocol implemented by the clinic for the treatment of athletes.

Participants were asked to walk around slowly while flexing and extending their elbows and fingers throughout both exposures. Before entering the chamber participants were asked to remove glasses, contact lenses and any jewellery or piercings. During exposure, participants wore a pair of shorts and nothing above the

waist. Participants also wore gloves, dry socks and shoes, a hat covering the ears and a mask to protect the nose and mouth.

3.5. Dietary control

Throughout the duration of all 3 studies, participants were asked to maintain their habitual diet but to avoid any nutritional supplements (e.g. protein, BCAA's and antioxidants). For the nutritional supplements provided as part of study 3, details are included within the relevant chapter. All data were collected in a non-fasted state. For study 3, food diaries were collected during the first and last week of data collection and then analysed to ensure there were no differences in dietary intake (total calories or macronutrients) between groups.

3.6. Statistical Analysis

Findings from all of the studies in this thesis are reported using inferential Magnitude Based Inference (MBI) statistics. MBI has been proposed as an alternative to traditional NHSTs that may allow researchers to understand the size of a true effect, rather than relying solely on significant versus non-significant outcomes (Batterham & Hopkins, 2006). MBI may be more useful in cases where sample sizes are small (as is common in sport science research) and can provide researchers, practitioners and athletes with an indication of the practical meaningfulness of the results (Hopkins, Marshall, Batterham, & Hanin, 2009). The selection of this method suits the applied nature of this thesis.

Confidence intervals (CI) and magnitude based inferences were calculated for each dependent variable using methods described by Batterham and Hopkins (2006). The smallest practically worthwhile effect for muscle function and blood parameters was the smallest standardised (Cohen) change in the mean: 0.2 times the between-subject SD for baseline values of all participants (Batterham & Hopkins, 2006). The smallest worthwhile change for muscle soreness, perceptions of recovery and

DALDA scores was a change in raw values of 1.0 (Hopkins, 2015). In order to account for large inter-individual differences in blood parameters, baseline values were used as a covariate. Qualitative descriptors relate to the likelihood of increased, trivial or decreased outcomes. Clinical inferences were based on threshold chances of harm and benefit of 0.5 and 25% respectively. In cases where the inference was unclear, a beneficial inference was reported where the odds ratio of benefit/harm was greater than 66.

In order to overcome heteroscedastic error, the analysis of dependent variables was conducted on log-transformed data (Nevill & Lane, 2007), except in the cases of muscle soreness, perceptions of recovery and DALDA. Interval scaling makes it inappropriate to log-transform data for these variables (Nevill & Lane, 2007) therefore analysis was conducted on raw values. Each dependent variable was analysed using a published spreadsheet by Hopkins (2015). Changes are reported as percentages for function variables, raw changes for perceptual variables and factor changes for blood markers. Effect sizes are reported in addition to magnitude based inferences, where 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large (Hopkins, Marshall, Batterham, & Hanin, 2009). Baseline values for all dependent variables are presented as raw mean ± SD. For changes over time and group comparisons, data are presented as mean difference ±confidence limit.

For the last few years, and particularly over the last 12 months, the appropriateness of MBI as a statistical approach has been called into question (Welsh & Knight, 2015). A paper published last year (Sainani, 2018) claimed that type I and II error rates are inflated with MBI compared to null hypothesis testing (in direct contrast to the claims made by proponents of the method), and that many error cases are incorrectly classified. Using code provided by Batterham and Hopkins (2016) Sainani (2018) ran hundreds of thousands of simulations whilst systematically

varying both the sample size (from 10 to 150 per group) and the threshold for harm/benefit (from 0.1 to 0.3 for sample sizes of 5 to 300 per group). Her findings demonstrate that type I error is greater for MBI than NHST for sample sizes of ~20 to 100 participants, but that below ~20 participants type I error rates are lower in MBI. Whilst this must obviously be taken into consideration, given that the sample sizes presented within this thesis are ≤ 11, MBI remains an appropriate and useful approach. The second issue highlighted by Sainani (2018) relates to the classification of errors; stating that any 'unclear' findings should be redefined as type II errors. When running simulations Sainani (2018) reclassified unclear findings as type II errors and this is reflected in the high type II error rate reported in the study. Whilst the decision from Batterham and Hopkins (2006) to report unclear findings 'free of the burden of type I and type II errors' may be problematic or 'incorrect' mathematically speaking, unclear results generated during MBI analyses are accompanied by an instruction to 'get more data'. As such, these findings are not classified as decisive or clear outcomes and no inference is given. Therefore, despite the criticisms raised in the literature it was determined that the use of MBI for the present investigation was appropriate.

Chapter 4

Recovery following a marathon: A comparison of cold water immersion, whole body cryotherapy and a placebo control

4.1. Introduction

Across both recreational and elite level sport, athletes regularly train or even compete multiple times a week. It is well documented that novel or exhaustive exercise, whether mechanical or metabolic in nature, can result in exercise induced muscle damage (EIMD) (Belcastro et al., 1998) and inflammation (Pyne, 1993). These physiological stresses can lead to reduced performance potential, likely as a result of increased muscle soreness (Cheung et al., 2003) and decreased muscle function (Christopher Byrne & Eston, 2002), as well as increased stiffness and swelling that can last for a number of days following the initial insult (Armstrong, 1984).

Where performance is a crucial consideration, the optimisation of recovery in between exercise bouts to minimise any negative impact on subsequent performance is vital (Barnett, 2006). Cryotherapy, either in the form of CWI or WBC, is becoming an increasingly popular recovery strategy employed by athletes (Hohenauer et al., 2015). Changes in physiological mechanisms resulting from a decrease in muscle and/or skin temperature include; reduced inflammation, analgesia, reductions in cardiovascular strain, decreased blood flow, reduced tissue metabolism, increased removal of muscle metabolites as well as neuromuscular, cardiovascular and hormonal changes (Ihsan, Watson, & Abbiss, 2016; Leeder, Gissane, van Someren, Gregson, & Howatson, 2012). It is likely that any performance effects resulting from a cryotherapy intervention could be attributed to one, or a combination, of these physiological phenomena.

Despite the growing body of literature, there is still a lack of clarity regarding the efficacy of CWI and WBC as recovery strategies. This may be due in part to the fact that the type and magnitude of physiological stress experienced following a bout of exercise is heavily dependent on the specific nature and duration of the exercise.

Evidence suggests that CWI can attenuate soreness (Bleakley et al., 2012; Hohenauer et al., 2015; Leeder et al., 2012) following a variety of exercise stressors but the effect on muscle function remains less clear (Bleakley et al., 2012; Hohenauer et al., 2015). However, there is conflicting evidence to demonstrate that CWI has no effect on soreness (Leeder et al., 2015) following repeated sprints. Similarly, WBC has been shown to attenuate soreness following metabolic and mechanical stress (Hausswirth et al., 2011), but recent evidence suggests that ambiguity remains in the literature (Costello et al., 2015). However, there is little evidence to support improvements in functional recovery (Bleakley, Bieuzen, Davison, & Costello, 2014). It is likely that the equivocal results are due to differences in temperature, timing of application, type of exercise stress and training status of participants (Minett & Costello, 2015).

The rise in popularity of WBC as an alternative to CWI may be explained in part by the capacity to attain far lower exposure temperatures, possibly offering enhanced benefits to recovery. It has been proposed that cryotherapy has the potential to limit inflammation by decreasing peripheral blood flow and therefore limiting migration of inflammatory cytokines to areas of structural damage (Mawhinney et al., 2017). However, although WBC produces greater temperature gradients for tissue cooling, the relatively poor thermal conductivity of air compared to water limits the potential for significant subcutaneous and core body cooling (Bleakley et al., 2014). This is supported by both Costello et al. (2014) and Mawhinney et al. (2017) who demonstrated that CWI exposure elicits greater reductions in skin and tissue temperature than WBC. Despite a growing body of literature, there are still relatively few studies that directly compare WBC and CWI (Abaïdia et al., 2016; Mawhinney et al., 2017). Further research is required to afford researchers better understanding of the circumstances under which either treatment is, or is not effective, and whether one intervention can offer any substantial benefit over the other.

To date only one study has directly compared the efficacy of CWI and WBC on functional recovery. Abaïdia et al. (2016) compared the effects of CWI (10 min at 10°C) and WBC (3 min at -110°C) on recovery following eccentric single-leg hamstring exercise. Their results demonstrated that there was a very likely moderate effect in favour of CWI for recovery of single and double leg CMJ compared to WBC 72 hours post exercise. Further, CWI elicited a moderate reduction in perceived soreness and a moderate increase in perception of recovery at 24 and 48 h post respectively. The authors concluded that CWI was more effective than WBC in enhancing recovery of CMJ 72 hours after exercise. However, the exercise stress utilised in this study lacks ecological validity, and there was no control group for comparison.

Given the trend for increasing use of WBC despite equivocal research relating to both CWI and WBC, it is pertinent to make direct comparisons between the two modalities to investigate whether one method can offer a considerable advantage over the other. There are currently only a handful of studies directly comparing WBC and CWI and methodological differences make it difficult to draw cross study comparisons; the need for specificity in post-exercise recovery strategies has previously been highlighted by Minett and Costello (2015) and Stephens et al. (2016).

Long duration endurance exercise such as marathon running results in alterations of a number of physiological and perceptual parameters including muscle soreness, muscle function, muscle damage (CK) and inflammation (CRP) (Hill et al., 2014; Shanely et al., 2013). Whilst a number of studies have utilised CWI as a means of rapid cooling in the treatment of exertional heatstroke following marathon performance (Casa et al., 2007; Mcdermott et al., 2009), there appears to be little evidence evaluating the efficacy of CWI or WBC on recovery following a marathon.

Therefore the aim of this study was to investigate the effects of CWI, WBC or a placebo intervention on both physiological and perceptual parameters of recovery in trained runners following the completion of a marathon run. It was hypothesised that cryotherapy would be more beneficial for perceptual recovery than the placebo intervention, but that functional recovery would be comparable across all treatments.

4.2. Methods

4.2.1. Participants

31 healthy male volunteers participated in this study (Table 4.1). Participants were trained endurance runners and had an expected completion time of 4.5 hours or less for a marathon. All participants were non-smokers with no history of recent illness or other disease. In the five days prior to the run and for the duration of the study, participants were asked to abstain from therapeutic treatments including massage and anti-inflammatory drugs, as well as any nutritional supplements. Participants were instructed to refrain from strenuous exercise (other than the marathon itself) for at least 2 days before each testing session.

4.2.2. Study design

Participants were randomly assigned into the placebo (n=10), CWI (n=11) or WBC (n=10) intervention group. On the first testing day and prior to the marathon run participants were familiarised with all testing procedures before baseline measures of all dependent variables (DVs) were recorded. Participants began their allocated treatment intervention within 15 minutes of cessation of exercise, and then provided a further blood sample for analysis. Repeat measurements of all DVs were recorded at 24 h and 48 h following completion of the run. Data collection took place over a number of months and environmental conditions were recorded for each marathon day. Participant characteristics, completion times and environmental conditions are included in table 4.1.

Table 4.1. Participant characteristics, completion times and environmental conditions.

	Age (yr)	Height (cm)	Body Mass (kg)	Marathon PB (hh:mm:ss)	Completion Time (hh:mm:ss)	Max Temp (ºC)
PL	41 ± 7	174.7 ± 8.6	75.9 ± 10.2	03:20:27 ± 00:25:19	03:40:18 ± 00:33:08	18.4 ± 3.7
CWI	41 ± 8	178.3 ± 7.6	79.2 ± 10.2	03:33:33 ± 00:27:10	03:43:05 ± 00:13:42	19.1 ± 4.2
WBC	38 ± 9	178.0 ± 5.2	77.8 ± 6.9	03:36:51 ± 00:18:48	03:59:06 ± 00:17:13	17.8 ± 1.8

Values are presented as mean ± SD
Max Temp refers to average maximum temperature recorded on the baseline testing days for each intervention

4.2.3. Exercise protocol

Participants completed the marathon in North London and were asked to pace the run as if it were a competitive race. The route was predominantly grass and unpaved footpaths, with some short concrete sections. Participants completed 9 laps of a 4.7 km loop which was marshalled at the start/finish point. Participants were allowed to consume fluids, electrolytes and/or food ad libitum during the run but were asked to avoid consuming any supplements containing branched chain amino acids, protein, antioxidants or caffeine. By using an outdoor route and self-selected pace, it was hoped that the run would more closely mimic a real world scenario than a treadmill based protocol.

4.2.4. Dependent variables

4.2.4.1. Perceived soreness

Please refer to section 3.3.2.

4.2.4.2. Daily analysis of the lifestyle demands of athletes (DALDA)

Participants completed a DALDA questionnaire on each testing day. Further detail is provided in section 3.3.3.

4.2.4.3. Peak torque and isometric contractions

Please refer to section 3.3.5.

4.2.4.4. Drop jump (DJ)

Please refer to section 3.3.7.

4.2.4.5. Blood sampling

Blood samples were collected in accordance with section 3.3.9. Whole blood samples were assessed using a blood counter (AcT diff 5 CP, Beckman Coulter, High Wycombe, UK) to correct values for changes in plasma volume. The manufacturer reports the coefficients of variation for this system as <2% for haematocrit and <1% for haemoglobin within the reportable range. Blood markers included CK-M, CRP, IL-6 and TNF-α and were all determined in duplicate.

4.2.4.5.1. CK-M

Please refer to section 3.3.9.1.

4.2.4.5.2. CRP

Please refer to section 3.3.9.2.

4.2.4.5.3. IL-6

Please refer to section 3.3.9.3

4.2.4.5.4. TNF-α

Please refer to section 3.3.9.4.

4.2.4.5.5. Correction for haemoconcentration

Bouts of acute exercise, such as long distance running, have been shown to produce a transient fluid shift out of the intravascular space, resulting in haemoconcentration (Kargotich, Goodman, Keast, & Morton, 1998). To minimise any confounding effects of the dynamic nature of plasma volume (Alis et al., 2015),

changes in plasma volume were assessed using the following equation; ΔPV (%) 100 × ((HBpre/HBpost) × (100−HTCpost)/(100−HTCpre) −1) where PV is plasma volume, HB is haemoglobin and HTC is haematocrit. All blood markers were then adjusted using the following equation; (Biomarker)c = (Biomarker)u × (1+ ΔPV (%) /100) where c is corrected and u is uncorrected.

4.2.5. Interventions

4.2.5.1. Placebo

As it was not possible to blind participants to their recovery intervention, a placebo, rather than a control group was used. The phytochemicals found in Tart Montmorency cherry juice have previously been shown to reduce inflammation and improve muscle recovery following a marathon (Howatson et al., 2010). Therefore, the placebo group was informed that they were taking a tart cherry juice supplement for 5 days before the run, the day of the run and for 2 days after (8 days in total). Participants consumed 2 x 30 ml per day of a prediluted fruit flavoured squash drink (Tesco, cherries and berries no added sugar squash, ~2 calories per 30ml serving) which did not contain any antioxidants or phytonutrients. Participants were asked to rest quietly for 10 minutes following completion of the run. It was hoped that the use of a placebo (sham) group would minimise associated placebo effects (i.e. effects of the treatment that were not related to the treatment itself) (McClung & Collins, 2007).

4.2.5.2. Cold water immersion (CWI)

Immediately after cessation of exercise participants sat in a mobile ice bath (iSprint Twin, iCool, Cranlea, UK) ensuring their lower limbs and iliac crest were fully immersed. For more information please refer to section 3.4.2.

4.2.5.3. Whole body cryotherapy (WBC)

The WBC group was exposed to 2 cold treatments in a cryotherapy chamber (CryoClinics, London, UK). For further details please refer to section 3.4.3.

4.2.6. Statistical analysis

All dependent variables were analysed using magnitude based inferences. Statistical analyses were conducted in accordance with the procedures outlined in section 3.6.

4.3. Results

The outcomes for changes over time as well as group comparisons for all parameters can be seen in tables 4.2 and 4.3. The marathon resulted in decreases in muscle function, increases in circulating CK, increases in perceptions of soreness and alterations in a number of blood borne markers of inflammation.

4.3.1. Perceptual responses

A summary of the statistical analyses for the effect of each intervention on perceptual responses can be seen in table 4.2.

4.3.1.1. Perceived muscle soreness

At baseline, soreness values were 1 ± 2, 1 ± 1 and 1 ± 1 (VAS 0-10) for PL, CWI and WBC respectively (Table 4.2). Perceptions of soreness increased in all groups from baseline to 24 h, and in the PL group from baseline to 48 h. WBC elicited possibly lower values compared to PL at 48 h, whilst all other group comparisons were trivial or unclear (Figure 4.1).

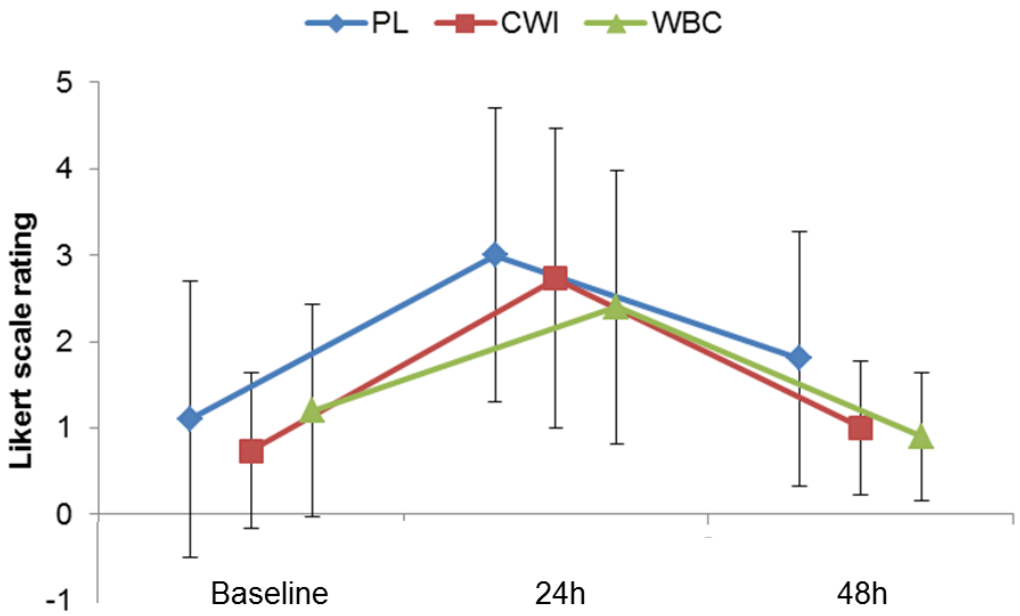

Figure 4.1. Changes in perceived muscle soreness. Data are presented as mean ± SD.

4.3.1.2. DALDA

At baseline, DALDA values were 4 ± 3, 1 ± 2 and 4 ± 4 scores marked as worse than normal for PL, CWI and WBC respectively. The number of scores marked 'worse than normal' increased in the CWI and PL group at 24 and 48 h, whereas the change was unclear at 24 h and reduced at 48 h for WBC. Scores were lower for WBC compared to CWI at all time points, and comparisons were unclear between WBC and PL. Scores were higher for CWI compared to PL at all time points.

4.3.2. Muscle function

A summary of the statistical analyses for the effect of each intervention on markers of muscle function can be seen in table 4.2.

4.3.2.1. Peak torque knee extension

At baseline, the peak torque knee extension values were 178.24 ± 28.41, 195.33 ± 29.92 and 203.72 ± 39.47 Nm for placebo, CWI and WBC respectively. Peak torque

decreased in all groups at both time points post marathon. WBC demonstrated a greater decrement at all time points in comparison to both CWI and PL (Figure 4.2).

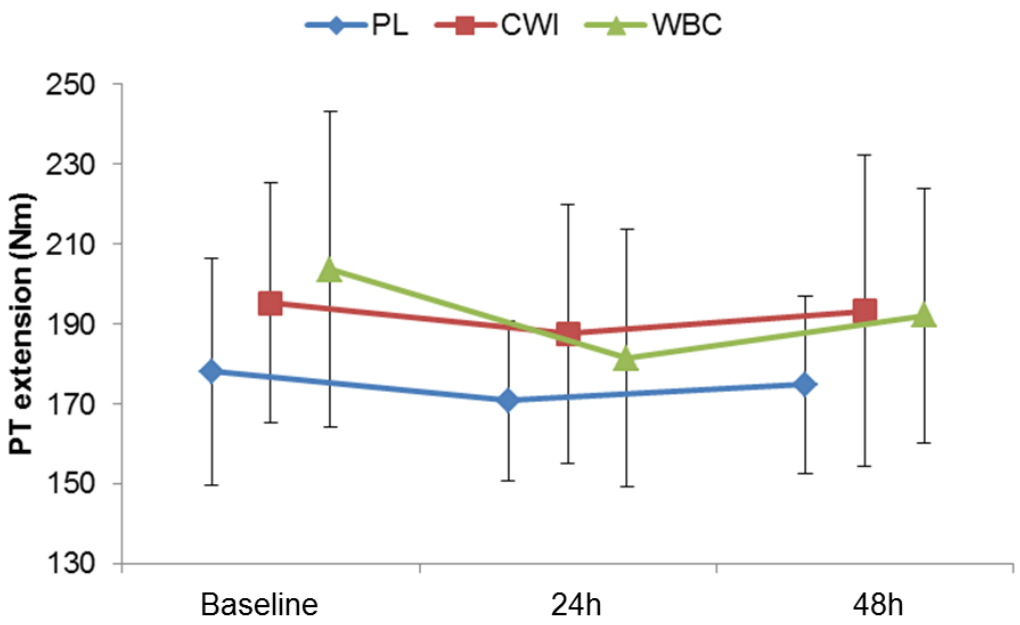

Figure 4.2. Changes in peak torque extension. Data are presented as mean ± SD.

4.3.2.2. MVIC

At baseline, MVIC values were 197.85 ± 51.15, 221.81 ± 37.48 and 228.60 ± 54.68 N for PL, CWI and WBC respectively. Changes in MVIC were unclear or trivial in the CWI and PL groups, whilst there was a decrease in the WBC group. Group comparisons revealed greater reductions for MVIC in WBC compared to both CWI and PL at all time points.

4.3.2.3. Reactive strength index (RSI)

At baseline, RSI values were 0.88 ± 0.21, 0.89 ± 0.30 and 1.03 ± 0.29 $m \cdot s^{-1}$ for PL, CWI and WBC respectively. RSI decreased in all groups from baseline to 24 h, with unclear or trivial changes from baseline to 48 h. WBC demonstrated a greater reduction compared to CWI and PL at all time points.

Table 4.2. Change over time and group comparison outcomes for muscle function and perceptual responses.

		Changes over Time Mean; ±CL Qualitative outcome			Effects Mean[a]; ±CL[b] Qualitative Outcome		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
Peak Torque (%)	B – 24h	-3.7; ±4.3 Small ↓*	-4.1; ±5.5 Small ↓*	-10.7; ±4.0 Small ↓***	-0.4; ±6.9 ↔ *	-7.3; ±5.5 Moderate ↓**	-6.9; ±6.4 Moderate ↓**
	B – 48h	-1.6; ±4.0 ↔*	-1.7; ±6.5 Trivial ↓*	-5.3; ±4.7 Small ↓*	-0.2; ±7.4 ↔*	-3.8; ±5.8 Small ↓*	-3.7; ±7.5 Small ↓*
MVIC (%)	B – 24h	1.7; ±5.5 ↔**	-0.7; ±5.2 ↔*	-10.1; ±3.3 Small ↓***	-2.4; ± 7.0 Small ↓*	-11.7; ±5.5 Large ↓***	-9.5; ±5.5 Moderate ↓**
	B – 48h	3.4; ±8.5 Unclear	1.1; ±5.5 ↔*	-8.0; ±6.5 Small ↓**	-2.2; ±9.3 Trivial ↓*	-11.0; ±9.2 Moderate ↓**	-9.0; ±7.8 Moderate ↓**
RSI (%)	B – 24h	-4.9; ±9.3 Trivial ↓*	-4.8; ±13.0 Trivial ↓*	-13.7; ±10.1 Small ↓*	0.1; ±16.1 ↔*	-9.2; ±13.2 Small ↓*	-9.3; ±15.6 Small ↓*
	B – 48h	2.9; ±12.5 Unclear	2.6; ±12.3 ↔*	-5.8; ±9.9 ↔**	-0.3; ±16.2 Trivial ↓*	-8.4; ±14.0 Small ↓*	-8.2; ±14.0 Small ↓*
Muscle Soreness	B – 24h	2; ±1 Moderate ↑**	2; ±1 Very large ↑**	1; ±1 Moderate ↑*	0; ±2 ↔*	-1; ±2 Unclear	-1; ±1 Unclear
	B – 48h	1; ±1 Small ↑*	0; ±0 ↔***	0; ±1 ↔***	0; ±1 ↔**	-1; ±1 Moderate ↓*	-1; ±1 ↔**
DALDA	B – 24h	2; ±3 Moderate ↑*	4; ±3 Large ↑***	0; ±2 Unclear	2; ±4 Small ↑*	-2; ±3 Unclear	-4; ±3 Moderate ↓**
	B – 48h	0; ±3 Trivial ↑*	1; ±3 Small ↑*	-2; ±2 Small ↓*	1; ±3 Small ↑*	-2; ±3 Unclear	-3; ±3 Moderate ↓**

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group.
[b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference
For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

4.3.3. Blood markers

A summary of the statistical analyses for the effect of each intervention on blood markers can be seen in table 4.3.

4.3.3.1. CK

At baseline, CK values were 31.2 ± 18.0, 25.0 ± 14.5 and 44.2 ± 70.4 U.L for PL, CWI and WBC respectively. CK increased in all groups at both time points post marathon. CK values were elevated in WBC compared to CWI, and in both cryotherapy interventions compared to PL at 24 h. All group comparisons from baseline to 48 h were unclear.

4.3.3.2. CRP

At baseline, CRP values were 1625 ± 3838, 586 ± 378 and 553 ± 573 ng.mL for PL, CWI and WBC respectively. CRP increased in all groups from baseline to 24 h and in the CWI and PL groups from baseline to 48 h. Values for WBC were elevated

compared to CWI and PL at 24 h, but reduced in the same comparisons at 48 h. CWI demonstrated smaller increases compared to PL at both time points.

4.3.3.3. IL-6

At baseline, IL-6 values were 42.60 ± 104.35, 75.93 ± 157.27 and 17.81 ± 33.51 pg/ml for PL, CWI and WBC respectively. IL-6 increased in all groups immediately post marathon, and remained elevated in the WBC group only at 24 h post. From baseline to post, values were reduced in WBC compared to CWI, increased in CWI compared to PL, and the comparison between WBC and PL was unclear. At 24 h, values for WBC were greater than CWI and PL, whilst the comparison between CWI and PL was unclear.

4.3.3.4. TNF-α

At baseline, TNF-α values were 52.5 ± 118.0, 327.6 ± 928.1 and 58.9 ± 119.4 pg/ml for PL, CWI and WBC respectively. TNF-α increased in all groups following the marathon. Comparisons between WBC and CWI were unclear at all time points, whilst values for WBC were higher than PL immediately post, and values for CWI were higher than PL at all time points.

Where there are large differences in baseline values between groups (CRP, IL-6 and TNF-α), this is attributed to one or two individuals who had values substantially greater than the normal range. However, as results are analysed as the difference between groups in change over time, these participants were not removed from the analysis.

Table 4.3. Change over time and group comparison outcomes for blood markers.

		Changes over Time Mean; x/÷ CL Qualitative outcome			Effects Mean[a]; x/÷ CL[b] Qualitative Outcome		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
CK	B – 24h	1.6; x/÷ 1.2 Moderate ↑****	2.5; x/÷ 2.2 Moderate ↑***	3.0; x/÷ 1.7 Moderate ↑****	1.4; x/÷2.2 Small ↑*	2.0; x/÷1.7 Moderate ↑***	1.4; x/÷2.4 Small ↑*
	B – 48h	1.5; x/÷ 1.2 Moderate ↑****	1.7; x/÷ 1.5 Small ↑***	1.5; x/÷ 1.5 Small ↑**	1.1; x/÷1.5 Unclear	1.1; x/÷1.6 Unclear	1.0; x/÷1.7 Unclear
CRP	B – 24h	23.2; x/÷ 1.5 Large ↑****	3.7; x/÷ 2.0 Large ↑***	26.2; x/÷ 1.4 Very large ↑****	0.2; x/÷2.2 Very large ↓****	1.5; x/÷1.6 Trivial ↑**	8.1; x/÷2.2 Very large ↑****
	B – 48h	13.5; x/÷ 1.4 Moderate ↑****	4.6; x/÷ 1.5 Large ↑****	0.9; x/÷ 9.4 Unclear	0.4; x/÷1.7 Moderate ↓***	0.1; x/÷9.8 Large ↓***	0.2; x/÷10.0 Moderate ↓**
IL-6	B – Post	2.7; x/÷ 1.5 Small ↑****	3.7; x/÷ 1.4 Small ↑****	2.8; x/÷ 1.7 Small ↑***	1.52; x/÷1.65 Small ↑**	0.96; x/÷1.88 Unclear	0.63; x/÷1.78 Small ↓**
	B – 24h	0.9; x/÷ 1.3 Unclear	0.9; x/÷ 1.4 Unclear	1.2; x/÷ 1.2 Trivial ↑**	0.92; x/÷1.50 Unclear	1.25; x/÷1.32 Moderate ↑**	1.36; x/÷1.46 Moderate ↑**
TNF-α	B – Post	1.0; x/÷ 1.2 ↔*	1.1; x/÷ 1.3 Trivial ↑*	1.0; x/÷ 1.1 ↔**	1.1; x/÷1.3 Trivial ↑*	1.1; x/÷1.2 Trivial ↑*	1.0; x/÷1.3 Unclear
	B – 24h	1.0; x/÷ 1.1 ↔**	1.2; x/÷ 1.3 Trivial ↑*	1.1; x/÷ 1.1 Trivial ↑*	1.2; x/÷1.3 Small ↑*	1.0; x/÷1.2 ↔*	0.9; x/÷1.3 Unclear
	B – 48h	1.1; x/÷ 1.1 Trivial ↑*	1.1; x/÷ 1.2 Trivial ↑*	1.0; x/÷ 1.2 Trivial ↑*	1.0; x/÷1.2 Trivial ↑*	1.0; x/÷1.2 Unclear	0.9; x/÷1.3 Unclear

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and ****most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – times and divide the mean by this number to obtain the 90% confidence limits for the true difference
For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

4.4. Discussion

The present study examined the efficacy of a single bout of WBC or CWI compared to a placebo intervention on markers of recovery in trained endurance athletes following a marathon run. The marathon led to modest alterations in muscle function, perceptions of muscle soreness and stress response symptoms, as well as increases in markers of muscle damage and inflammation. In terms of comparison between the different interventions, WBC was less effective for recovery of muscle function compared to CWI, with both cryotherapy groups being detrimental in comparison to the placebo. WBC reduced stress response symptoms and muscle soreness in comparison to CWI and the placebo, respectively. Both cryotherapy interventions led to greater increases in CK than the placebo, with CWI attenuating the response at 24 h compared to WBC. There was little evidence of the cryotherapy interventions limiting inflammation based on IL-6 and TNF-α, and in

some cases led to greater increases. However, cryotherapy led to the attenuation of increases in CRP.

WBC was detrimental to recovery of peak torque compared to both placebo and CWI at 24 and 48 h; there were no differences between CWI and placebo. These results suggest that CWI may offer benefits compared to WBC for the recovery of peak torque, but that it is no more effective than a placebo intervention. In the case of MVIC and RSI, WBC led to greater decrements than both CWI and placebo, and CWI was also less effective than the placebo for recovery. The novel finding that cryotherapy was less effective for recovery in comparison to a placebo could be linked to an enhanced inflammatory response evidenced by an increase in pro-inflammatory markers (Hausswirth et al., 2011). Some research suggests that cold exposure such as CWI has the potential to cause adverse effects that are interpreted by the body as noxious stimuli (Machado et al., 2016). Although muscle temperature was not recorded in the present study, it is possible that both an 8°C CWI exposure and a -85°C WBC exposure resulted in reductions of skin and/or muscle temperature that elicited a stress response in the body, which may explain the deleterious effects of cryotherapy in this case.

The present study's results contrast with those from White, Rhind and Wells (2014) who reported that CWI (10 min or 30 min at 10°C) facilitated restoration of drop jump performance following high-intensity sprint exercise. This study suggests that immersion at 8°C is less effective than a placebo intervention for functional recovery after endurance exercise. The use of a placebo group in place of a control could explain the findings; research suggests that many of the hypothesised physiological benefits surrounding CWI are at least partly placebo related (Broatch et al., 2014). Broatch and colleagues (2014) found that CWI was no more effective than a placebo immersion protocol at improving muscle recovery following high intensity exercise. They suggested that effective deception of participants is critical when

using a placebo and that treatment belief is a powerful element (Beedie et al., 2017). Anecdotal evidence from the present study suggests that the placebo was administered effectively, and that participants believed in its efficacy.

Differences in muscle soreness between WBC and CWI were either trivial or unclear. These results are in contrast to Abaïdia and colleagues (2016) who found that CWI resulted in lower soreness scores 48 h post eccentric exercise in comparison to WBC. These differences may be explained by the warmer WBC protocol utilised in the present study (-85° versus -110° C), potentially indicating that warmer WBC temperatures are more effective at alleviating perceptions of soreness after exercise, compared to 'extreme cold' exposures (Machado et al., 2016). Alternatively, the use of a novel high intensity eccentric biased exercise protocol may have produced greater structural damage, secondary inflammation and stimulation of pain receptors than that seen in the present study (Clarkson & Hubal, 2002; Scholz & Woolf, 2002). The finding that CWI was not effective at reducing soreness compared to the placebo is in contrast to the majority of previous research on the topic. An immersion temperature of 8°C may have been suboptimal; exposure temperatures of between 10 and 15°C are more commonly cited in the literature (Machado et al., 2016) and appear to be effective at reducing muscle soreness post exercise. Secondly, few studies investigating the influence of CWI on recovery have effectively blinded participants to their intervention group. The use of a placebo in the current study may negate some of the positive expectance effects attributable to the placebo effect (Broatch et al., 2014; McClung & Collins, 2007) and therefore offer a more robust examination of the effectiveness of CWI and WBC interventions used post exercise.

Cryotherapy appeared to exacerbate structural muscle damage, assessed via circulating CK. Between baseline and 24 h CK was elevated after WBC compared to CWI, and both cryotherapy treatments led to increased circulating CK compared

to the placebo. A recent study from Abaïdia and colleagues (2016) demonstrated a very likely large effect for CK in favour of CWI compared to WBC 24 h post exercise, supporting the present study's indication that CWI may offer additional benefits over WBC for the attenuation of CK 24 h post exercise. Cryotherapy led to greater increases in leucocytes following the marathon (unreported data). It is plausible that leucocytosis lead to an increased breakdown of the sarcolemma that increased the efflux of CK in the cryotherapy groups in comparison to the placebo.

Cryotherapy is proposed to potentiate anti-inflammatory actions by decreasing peripheral blood flow (Mawhinney et al., 2017). The results from the present study do not support this hypothesis; despite WBC attenuating IL-6 compared to CWI from baseline to post, when compared to the placebo, CWI resulted in increased IL-6, and WBC resulted in an unclear comparison. WBC and CWI increased the TNF-α response with no clear differences between the cryotherapy groups. As previously stated, this may be attributed to a cold related stress response (Machado et al., 2016), which may in turn increase markers of inflammation. However, CRP was the only marker where cryotherapy treatment had a positive influence compared to placebo. The seemingly equivocal results could be explained in part by different time courses of the markers; IL-6 tends to peak immediately post exercise (Bernecker et al., 2013; Clifford et al., 2017; Mündermann et al., 2016), whereas CRP normally continues to increase until 24 h post exercise (Howatson et al., 2010). As such, cryotherapy applied post exercise may have been ineffective at attenuating increases in IL-6 but could positively impact upon the recovery of CRP.

Potential limitations of the current study should also be addressed. The cryotherapy chamber utilised during data collection was located a short distance off site, and as such, there may have been slight inconsistencies in the timing of the post exercise blood sample for participants in the WBC group compared to CWI and placebo. It is possible that the delay in sampling could have resulted in inflated pro-inflammatory

values in the WBC group for the post exercise samples. However, the factor-fold increases in IL-6 and TNF-α were still greater in the CWI group compared to WBC immediately post. Secondly, the WBC treatment temperature in the present study (-85°C) was warmer than normally reported in the literature (-110 to -140°C); dictated by the minimum operating temperature of the cryotherapy chamber. Therefore, although the results reported here add to the current body of literature, the results cannot be generalised to colder exposure temperatures.

In terms of comparison between cryotherapy modalities, with the exception of DALDA scores at 24 and 48 h, CRP at 48 h and IL-6 immediately following the marathon, WBC demonstrated an unclear, or undesirable impact on all markers at all time points compared to CWI. These findings contradict the widely held assumption that WBC can elicit enhanced recovery benefits when compared to more traditional cryotherapy applications such as CWI.

Secondly, with the exception of soreness at 48 h for WBC and CRP (24 and 48 h for CWI and 48 h for WBC) the implementation of a cryotherapy intervention resulted in unclear, trivial or undesirable effects for every outcome measure when compared to the placebo intervention. This lends further weight to the suggestion that therapeutic effects attributed to cryotherapy protocols could be a product of the placebo effect. Therefore, this highlights the need for future research to implement effective placebo interventions in place of control groups, or to at least take into consideration a measure of treatment belief when comparing different intervention strategies.

4.5. Conclusion

The overall aim of the thesis was to add to the current body of knowledge relating to the effects of acute and habitual cryotherapy exposure on recovery and adaptation following strenuous exercise, using placebo-controlled study designs. In terms of the specific thesis aims, this study addressed aim 1 (to establish whether

cryotherapy is any more effective than a placebo intervention for recovery following an acute bout of strenuous exercise), 2 (to determine whether WBC is any more effective than CWI) and 3 (to establish whether exercise mode influences the impact of cryotherapy on recovery). The findings from this study demonstrated that neither cryotherapy intervention was more effective than a placebo for improving functional recovery or perceptions of training stress following prolonged endurance exercise. However, WBC was more effective at reducing muscle soreness and perceptions of training stress compared to CWI. Further research is warranted to investigate the impact of CWI versus WBC on recovery following different exercise stresses, and to further examine the potential contribution of a placebo effect.

Chapter 5

Recovery following resistance exercise: A comparison of cold water immersion, whole body cryotherapy and a placebo control

5.1. Introduction

The first study of this thesis examined the efficacy of CWI and WBC on markers of recovery following prolonged endurance exercise. The findings demonstrated that CWI was superior to WBC for the recovery of functional markers post exercise, but interestingly, and possibly more importantly, neither cryotherapy intervention was superior to the placebo for recovery of functional markers or perceptions of training stress following the marathon. Additionally, the application of cryotherapy post exercise actually exacerbated the CK, IL-6 and TNF-α response at certain time points compared to the placebo.

Despite the growing popularity of WBC there is still very little available research investigating its efficacy following strenuous exercise. The findings from the first study of this thesis demonstrated that WBC negatively impacted the recovery of muscle function compared to CWI following a trail marathon. However, Minett and Costello (2015) highlight the need for specificity in the prescription of recovery interventions. It is well known that the mechanisms of muscle damage differ depending on the nature of the exercise stress (Armstrong et al., 1991); whilst long duration exercise without a significant eccentric component is likely to result in predominantly metabolic damage (Tee et al., 2007), resistance exercise can result in the breakdown of structural elements of muscle tissue and potentially greater functional perturbations. Therefore, the effectiveness of any recovery modality should be examined in relation to different exercise modes.

To date there appears to be only 1 study that has compared the efficacy of CWI and WBC on markers of recovery following strenuous exercise. Abaïdia and colleagues (2016), utilised an eccentric muscle damaging protocol and their results showed that CWI was more effective for accelerating both functional and perceptual recovery post exercise compared to WBC. Whilst the findings add to the current body of

literature, the unilateral eccentric exercise protocol used has little real world applicability to sports performance and therefore lacks ecological validity. Furthermore, muscle damaging exercise was carried out unilaterally, with conclusions about effectiveness based on bilateral vertical jump performance. A recent study from Hohenauer and colleagues (2018) evaluated the effect of PBC and CWI on recovery following 5 x 20 drop jumps. Their findings suggested that although there was no treatment effect for soreness or functional recovery, there was a greater physiological response (assessed via cutaneous vascular conductance, thigh muscle oxygen saturation and lower extremity skin temperature) for CWI compared to PBC. These findings are supported by Mawhinney et al. (2017) who demonstrated that limb blood flow is reduced to a greater extent following CWI than WBC. Presently, there do not appear to be any other studies directly comparing the effectiveness of the two different cryotherapy modalities on functional recovery after resistance exercise.

The importance of resistance exercise as an adjunct to more traditional sport specific skills training is becoming more evident in competitive sport (Bartolomei, Hoffman, Merni, & Stout, 2014). Progressive, structured, heavy resistance training is no longer solely used by bodybuilders and weightlifters but also by team sport players, dancers, gymnasts and swimmers (Crowley, Harrison, & Lyons, 2017; Dowse, McGuigan, & Harrison, 2017). Therefore, it is pertinent to assess the influence of cryotherapy on markers of recovery following resistance training. Moreover, there is still scope to explore whether WBC and CWI exposures elicit different physiological responses and time courses of recovery (Hayter, Doma, Schumann, & Deakin, 2016) following strenuous exercise.

Furthermore, there appears to be increasing evidence that many of the therapeutic effects attributed to cryotherapy treatment may be due to a placebo effect (Broatch

et al., 2014), and this notion is supported by the findings from the first study of this thesis. Currently, the vast majority of cryotherapy studies have been conducted using a control group. Therefore, there is a need for future investigations to evaluate cryotherapy treatments in comparison to an effectively administered placebo intervention, rather than a control.

Hence, the main aim of this study was to compare the efficacy of CWI, WBC or a placebo on recovery following strenuous resistance exercise, in order to try and address the current disparity in the literature. A further aim of this study was to use a holistic approach, encompassing performance, perceptual and blood borne markers, to establish whether either cryotherapy modality is any more effective than a placebo intervention following resistance exercise. It was hypothesised that CWI would be more beneficial for recovery than WBC, but that neither intervention would be more efficacious than a placebo treatment.

5.2. Methods

5.2.1. Participants

A convenience sample of twenty four healthy male volunteers participated in this study (Table 5.1). Participants had no previous experience of cryotherapy and were required to have at least 12 months experience of strength training. All participants were non-smokers with no history of recent illness or lower limb injury. For 72 h prior to the baseline testing day and for the duration of the study, participants were asked to refrain from any additional strenuous exercise, and abstain from therapeutic treatments including massage and anti-inflammatory drugs, as well as any nutritional supplements.

Table 5.1. Participant characteristics

	n	Age (y)	Height (m)	Mass (kg)	Predicted 1RM (kg)	Lean mass (kg)	Back squat 1RM/Lean mass	sRPE
PL	8	26 ± 5	1.80 ± 0.04	84.88 ± 13.81	125 ± 19.6	63.83 ± 7.51	1.95 ± 0.15	7.50 ± 1.41
CWI	8	22 ± 3	1.79 ± 0.05	84.39 ± 14.22	126 ± 21.3	66.56 ± 6.29	1.89 ± 0.22	7.63 ± 1.41
WBC	8	27 ± 8	1.71 ± 0.06	70.92 ± 10.20	120 ± 46.1	58.44 ± 6.49	2.04 ± 0.69	7.50 ± 1.07

Values are presented as mean ± SD
1RM, 1 Repetition Maximum; sRPE, session rate of perceived exertion

5.2.2. Study design

Participants were matched into the placebo, CWI or WBC intervention group based on a ratio of their predicted 1RM back squat to lean mass assessed via DXA scan (fan beam, Lunar Prodigy 4, GE Medical Systems, Lunar, Madison, WI, USA) (Roberts, Raastad, et al., 2015). Participants were familiarised with all testing procedures at least 72 h before the baseline session. At baseline, measures of all dependent variables were recorded before completion of the training session. Immediately after the training session a further blood sample was collected, and within 15 min participants commenced their allocated recovery intervention. Participants were also required to give blood samples at 60 and 120 min post intervention. Participants returned to the laboratory to repeat measurements of all dependent variables at 24, 48 and 72 h following completion of the resistance training session.

5.2.3. Calculation of repetition maximums (RMs)

Please refer to section 3.3.4.

5.2.4. Exercise protocol

For the resistance training session, all exercises were performed at 80% of the predicted 1RM for each exercise. The training session comprised 4 sets of 6 reps of back squats, 4 sets of 8 reps of split squats, 4 sets of 8 reps of hip thrusts and 4 sets of 8 reps of Romanian deadlifts. This represented a total volume of 120

repetitions which is comparable to other studies utilising resistance exercise and/or plyometrics to investigate recovery (Byrne & Eston, 2002; Jakeman, Byrne, & Eston, 2010), but offers a more ecologically valid exercise model to examine the efficacy of cryotherapy (Minett & Costello, 2015). Fifteen minutes after cessation of exercise, participants were asked to record a session RPE (Day, Mcguigan, Brice, & Foster, 2004) (Table 5.1.)

5.2.5. Dependent variables

5.2.5.1. Perceived soreness

Perceptions of muscle soreness were recorded as described in section 3.3.2.

5.2.5.2. Daily analysis of the lifestyle demands of athletes (DALDA)

Stress reaction symptoms were recorded using the DALDA questionnaire. For further detail, please refer to section 3.3.3.

5.2.5.3. Peak torque and isometric contractions

For further detail please refer to section 3.3.5.

5.2.5.4. Reactive strength index (RSI)

RSI data was derived from drop jump data which was collected as described in section 3.3.7.

5.2.5.5. Countermovement jump (CMJ)

CMJs were performed in accordance with the protocol described in section 3.3.8. Raw data was analysed in accordance with Chavda et al. (2017), and peak jump height values from each testing session were used for statistical analysis.

5.2.5.6. Isometric squat

Please refer to section 3.3.6.

5.2.5.7. Blood sampling

Blood samples were collected in line with the procedures outlined in section 3.3.9. Blood samples were taken at baseline (CK-M, IL-6, CRP & TNFα), immediately post training (IL-6 and TNFα), 60 and 120 min post intervention (IL-6 and TNFα), 24 (CK-M, IL-6, CRP & TNFα), 48 and 72 h post (CK-M, CRP & TNFα) post intervention (Leeder et al., 2014).

5.2.5.7.1. CK-M

Please refer to section 3.3.9.1.

5.2.5.7.2. IL-6

Please refer to section 3.3.9.3.

5.2.5.7.3. CRP

Please refer to section 3.3.9.2.

5.2.5.7.4. TNF-α

Please refer to section 3.3.9.4.

5.2.6. Interventions

5.2.6.1. Placebo

As it was not possible to blind participants to their recovery intervention, a placebo, rather than a control group was used. Branched chain amino acids are commonly used by athletes and have been shown to accelerate recovery following resistance training (Norton & Layman, 2006). Therefore, participants in the placebo group were

given a cornstarch pill and informed that they were taking a branched chain amino acid supplement after the training session. Participants were asked to rest quietly for 10 min following completion of the training session. It was hoped that the use of a placebo (sham) group would minimise associated placebo effects (i.e. effects of the treatment that were not related to the treatment itself) (McClung & Collins, 2007).

5.2.6.2. Cold water immersion (CWI)

Immediately after cessation of exercise participants sat in a mobile ice bath (iSprint Twin, iCool, Cranlea, UK) ensuring their lower limbs and iliac crest were fully immersed. For more information please refer to section 3.4.2. This protocol is comparable to those utilised in other single exposure studies examining the effects of CWI on various measures of recovery (Ascensão, Leite, Rebelo, Magalhães, & Magalhães, 2011; Roberts et al., 2014).

5.2.6.3. Whole body cryotherapy (WBC)

The WBC group was exposed to 2 cold treatments in a cryotherapy chamber (BOC, London, UK). For further details please refer to section 3.4.3.

5.2.7. Statistical analysis

All dependent variables were analysed using magnitude based inferences. Statistical analyses were conducted in accordance with the procedures outlined in section 3.6.

5.3. Results

The outcomes for changes over time as well as group comparisons for all parameters can be seen in tables 5.2 and 5.3. The resistance exercise session resulted in increased perceptions of soreness and stress reaction symptoms,

decreases in muscle function, and increases in markers of structural damage and inflammation.

5.3.1. DALDA

At baseline, DALDA values were 4 ± 6, 1 ± 1 and 2 ± 2 scores marked as worse than normal for placebo, CWI and WBC respectively. Scores marked worse than normal peaked at 24 h for the placebo group and at 48 h for both cryotherapy groups. CWI demonstrated greater increases compared to placebo at all time points. Scores were greater for WBC compared to the placebo at 48 h, but demonstrated a beneficial effect compared to the placebo at 24 h. All other group comparisons were trivial or unclear.

5.3.2. Perceived soreness

At baseline, soreness values were 1 ± 1, 1 ± 1 and 2 ± 2 (VAS 0-10) for placebo, CWI and WBC respectively. Perceptions of soreness increased in all groups; scores remained elevated in the placebo and CWI groups, but returned to baseline levels in the WBC group at 72 h post. WBC elicited smaller increases compared to both placebo and CWI at 24 h, but comparisons were unclear at 48 and 72 h post. CWI demonstrated a trivial effect compared to placebo at all time points.

Table 5.2. Change over time and group comparisons for perceptual markers

		Changes Mean; ± CL Qualitative outcome			Effects Mean[a]; ± CL[b] Qualitative Outcome		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
DALDA	B – 24h	0.38; ± 3.3 Very large ↑*	1.5; ± 0.9 Large ↑**	-0.38; ± 0.9 ↔**	1.12 ± 3.4 Small ↑*	-0.76 ± 3.4 Unclear	-1.88 ± 1.2 Moderate ↓**
	B – 48h	-0.25; ± 2.4 Unclear	1.63; ± 1.1 Large ↑**	0.5; ± 2.5 Small ↑*	1.88 ± 2.6 Moderate ↑*	0.75 ± 3.2 Small ↑*	-1.13 ± 2.6 Unclear
	B – 72h	-1.13; ± 2.8 Unclear	0.13; ± 0.2 ↔****	-0.75; ± 1.7 Unclear	1.26 ± 2.8 Small ↑*	0.38 ± 3.1 ↔*	-0.88 ± 1.7 Unclear
DOMS	B – 24h	3.88; ± 1.4 Very large ↑****	3.75; ± 1.4 Very large ↑****	0.63; ± 2.1 Small ↑*	-0.13 ± 1.8 ↔*	-3.25 ± 2.4 Large ↓**	-3.12 ± 2.1 Large ↓**
	B – 48h	3.50; ± 1.4 Very large ↑***	4.00; ± 1.8 Very large ↑***	0.88; ± 2.4 Small ↑*	0.50 ± 2.1 ↔*	-2.62 ± 2.6 Unclear	-3.12 ± 2.8 Unclear
	B – 72h	1.63; ± 1.1 Large ↑**	1.63; ± 1.6 Large ↑**	-0.25; ± 2.0 ↔*	0 ± 1.8 ↔*	-1.88 ± 2.2 Unclear	-1.88 ± 2.4 Unclear

CL, confidence limit. Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

5.3.3. Peak torque and isometric contractions

5.3.3.1. MVIC 90°

At baseline, MVIC values at 90° were 273.60 ± 57.76, 255.00 ± 63.17 and 240.59 ± 69.74 N for placebo, CWI and WBC respectively. MVIC was reduced at all time points in all groups. CWI demonstrated greater decrements compared to placebo at 24 and 48 h post, whilst WBC was trivial compared to placebo at 24 h. Comparisons between CWI and WBC were unclear at all time points.

5.3.3.2. Peak torque 60°·s^{-1}

At baseline, peak torque values at 60°·s^{-1} were 223.54 ± 50.65, 207.18 ± 38.85 and 194.98 ± 37.61 Nm for placebo, CWI and WBC respectively. Changes in peak torque values at 60°·s^{-1} for the placebo group were trivial at all time points, but demonstrated a decrease in both cryotherapy groups between baseline and 24 h, and decreases or trivial changes between baseline and 48 h and baseline and 72 h.

Group comparisons demonstrated that at 24 h, values for both CWI and WBC were reduced compared to the placebo group, as a result of trivial changes in the placebo group.

5.3.4. RSI

At baseline, RSI values were 1.80 ± 0.28, 2.20 ± 0.31 and 2.07 ± 0.31 $cm \cdot s^{-1}$ for placebo, CWI and WBC respectively. For the placebo and CWI groups, all changes over time demonstrated decreased or unclear effects, and for WBC, there was a possible improvement at 24 h post, but a decrease at both 48 and 72 h post. Cryotherapy was unclear, or less effective compared to placebo at all time points, with the exception of WBC at 24 h which showed a likely improvement.

5.3.5. CMJ

At baseline, CMJ height values were 0.35 ± 0.06, 0.34 ± 0.04 and 0.40 ± 0.05 m for placebo, CWI and WBC respectively. The training bout resulted in decreased CMJ performance for all groups at all time points. The greatest decrements in performance were evident at 24 h post for placebo and WBC, and at 48 h post for CWI. In terms of group comparisons, WBC demonstrated a greater decrement compared to CWI at 24 h, but unclear, and trivial effects at 48 and 72 h respectively. When compared to the placebo intervention, CWI showed greater decrements at 48 and 72 h, whilst WBC showed greater decrements at both 24 and 72 h post.

5.3.6. Isometric squat

5.3.6.1. Isometric peak force

At baseline isometric peak force values were 1620.43 ± 330.01, 1693.36 ± 398.76 and 1763.01 ± 682.02 Nm for placebo, CWI and WBC respectively. Peak performance perturbations were evident at 48 h post for all groups. All group comparisons at 24 and 72 hours were either unclear or trivial. However, at 48 h CWI

showed a reduction compared to placebo, and WBC was improved compared to CWI and placebo.

5.3.6.2. RFD 100-200ms

At baseline, RFD values between 100 and 200 ms were 4866.78 ± 1889.46, 5022.59 ± 1081.36 and 4135.02 ± 1756.94 $Nm \cdot s^{-1}$ for placebo, CWI and WBC respectively. Decrements in performance were most pronounced at 24 h for WBC, and at 48 h for placebo and CWI. WBC demonstrated an improvement compared to CWI and placebo at 48 h, but comparisons were unclear at 72 h. WBC demonstrated a reduction in performance compared to the placebo at 24 h, and performance in the CWI group was reduced compared to the placebo at all time points.

Table 5.3. Change over time and group comparisons for functional markers

		Changes Mean; ±CL Qualitative outcome			Effects Mean[a]; ±CL[b] Qualitative Outcome		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
MVIC 90°	B – 24h	-9.0; ±6.0 Small ↓**	-19.4; ±16.3 Moderate ↓**	-10.4; ±9.0 Small ↓**	-10.4; ±17.0 Moderate ↓*	-1.4; ±10.2 ↔*	9.0; ±17.8 Unclear
	B – 48h	-12.5; ±6.6 Small ↓***	-20.1; ±17.0 Moderate ↓**	-12.1; ±11.8 Small ↓**	-7.6; ±17.6 Small ↓*	0.4; ±12.9 Unclear	8.0; ±19.5 Unclear
	B – 72h	-13.6; ±9.5 Moderate ↓**	-13.9; ±18.3 Small ↓ **	-5.1; ±10.6 Trivial ↓*	-0.3; ±19.7 Unclear	8.5; ±13.3 Unclear	8.8; ±20.0 Unclear
PT 60 deg·s^{-1}	B – 24h	-2.6; ±5.0 Trivial ↓*	-9.4; ±9.9 Small ↓**	-11.8; ±4.7 Moderate ↓***	-6.8; ±10.6 Moderate ↓*	-9.2; ±6.4 Moderate ↓**	-2.4; ±10.5 ↔*
	B – 48h	0.2; ±5.7 ↔**	-12.2; ±8.6 Moderate ↓**	-1.0; ±6.2 ↔*	-12.4; ±9.7 Large ↓**	-1.2; ±7.9 ↔*	11.2; ±10.0 Unclear
	B – 72h	-0.2; ±7.5 ↔*	-1.1; ±8.0 Trivial ↓*	-2.3; ±6.2 Trivial ↓*	-0.9; ±10.3 ↔*	-2.1; ±9.1 ↔*	-1.2; ±9.5 ↔*
RSI	B – 24h	-6.8; ±11.4 Small ↓*	-23.6; ±8.6 Large ↓****	4.4; ±5.3 Small ↑*	-16.8; ±13.3 Large ↓***	11.2; ±12.2 Unclear	28.0; ±9.6 Very large ↑****
	B – 48h	5.6; ±12.2 Unclear	-16.7; ±6.4 Moderate ↓****	-9.1; ±6.6 Small ↓**	-22.3; ±13.2 Large ↓***	-14.7; ±13.3 Moderate ↓**	7.6; ±8.6 Unclear
	B – 72h	-1.7; ±13.6 Unclear	-4.5; ±11.9 Small ↓*	-4.3; ±6.7 Small ↓*	-2.8; ±16.9 Unclear	-2.6; ±14.5 Trivial ↓*	0.2; ±12.9 Unclear
CMJ	B – 24h	-5.1; ±5.7 Small ↓*	-6.3; ±6.8 Small ↓**	-9.1; ±6.3 Small ↓**	-1.2; ±8.3 ↔*	-4.0; ±7.8 Small ↓*	-2.80; ±8.5 Small ↓*
	B – 48h	-3.8; ±4.9 Trivial ↓*	-7.6; ±10.2 Small ↓**	-5.7; ±4.3 Small ↓**	-3.80; ±10.8 Small ↓*	-1.90; ±6.0 ↔*	1.90; ±10.6 Unclear
	B – 72h	-0.4; ±6.7 ↔*	-4.3; ±9.1 Small ↓*	-5.3; ±5.7 Small ↓**	-3.90; ±10.6 Small ↓*	-4.90; ±8.1 Small ↓*	-1.0; ±10.1 ↔*
ISO PF	B – 24h	-13.2; ±11.3 Small ↓**	-14.4; ±12.1 Moderate ↓**	-3.2; ±10.4 Trivial ↓*	-1.20; ± 15.5 ↔*	10.0; ±14.4 Unclear	11.2; ±14.9 Unclear
	B – 48h	-18.8; ±8.1 Moderate ↓***	-29.8; ±17.9 Large ↓***	4.9; ±6.3 Trivial ↑*	-11.0; ±18.9 Moderate ↓*	23.7; ±9.6 Large ↑****	34.7; ±18.6 Very large ↑***
	B – 72h	-14.4; ±7.7 Moderate ↓***	-14.4; ±15 Moderate ↓**	-2.4; ±12.8 Trivial ↓*	0.0; ±16.1 Unclear	12.0; ±14.2 Unclear	12.0; ±18.4 Unclear
RFD 100-200	B – 24h	-3.0; ±7.1 ↔**	-23.7; ±13.6 Moderate ↓***	-23.1; ±17.2 Small ↓**	-20.7; ±14.7 Moderate ↓**	-20.1; ±18.0 Moderate ↓**	0.6; ±20.5 Unclear
	B – 48h	-20.3; ±12.4 Small ↓**	-35.2; ±20 Large ↓***	18.1; ±15.3 Small ↑**	-14.9; ±22.2 Moderate ↓*	38.4; ±18.3 Large ↑***	53.3; ±23.5 Very large ↑****
	B – 72h	-11.7; ±18.4 Small ↓*	-16.3; ±16.6 Moderate ↓**	12.0; ±15.9 Small ↑*	-4.6; ±23.2 Small ↓*	23.7; ±22.7 Unclear	28.3; ±21.5 Unclear

CL, confidence limit. Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

5.3.7. Bloods

5.3.7.1. CK-M

At baseline, CK-M values were 79.7 ± 27.6, 145.7 ± 184.8 and 253.2 ± 249.9 U/L for placebo, CWI and WBC respectively. Increases were most pronounced at 24 h in all

groups (most likely very large (4.66; x/÷1.21), most likely large (4.02; x/÷1.63) and most likely moderate (4.63; x/÷1.41) increases for PL, CWI and WBC respectively), and had not returned to baseline levels by 72 h in any group. Comparisons for the CWI group were unclear compared to placebo at 24 and 48 h, but demonstrated a possibly small increase at 72 h (1.20; x/÷1.45). WBC demonstrated a trivial effect compared to placebo at 24h, but a possibly moderate (1.44; x/÷1.69) and likely large (1.57; x/÷1.40) increase at 48 and 72 h respectively. For comparison between cryotherapy modalities, WBC demonstrated a possibly small (1.15; x/÷1.73), likely moderate (1.61; x/÷1.94), and possibly moderate (1.31; x/÷1.51) increase compared to CWI at 24, 48 and 72 h respectively.

5.3.7.2. IL-6

At baseline, IL-6 values were 778.4 ± 2015.8, 25.9 ± 27.2 and 16.5 ± 27.8 pg/ml for placebo, CWI and WBC respectively. Change over time revealed trivial effects for all groups at all time points with the exception of WBC immediately- and 120 min post, where there were possible small increases (1.78; x/÷1.78 and 1.76; x/÷1.53 respectively). All group comparisons were trivial at all time points.

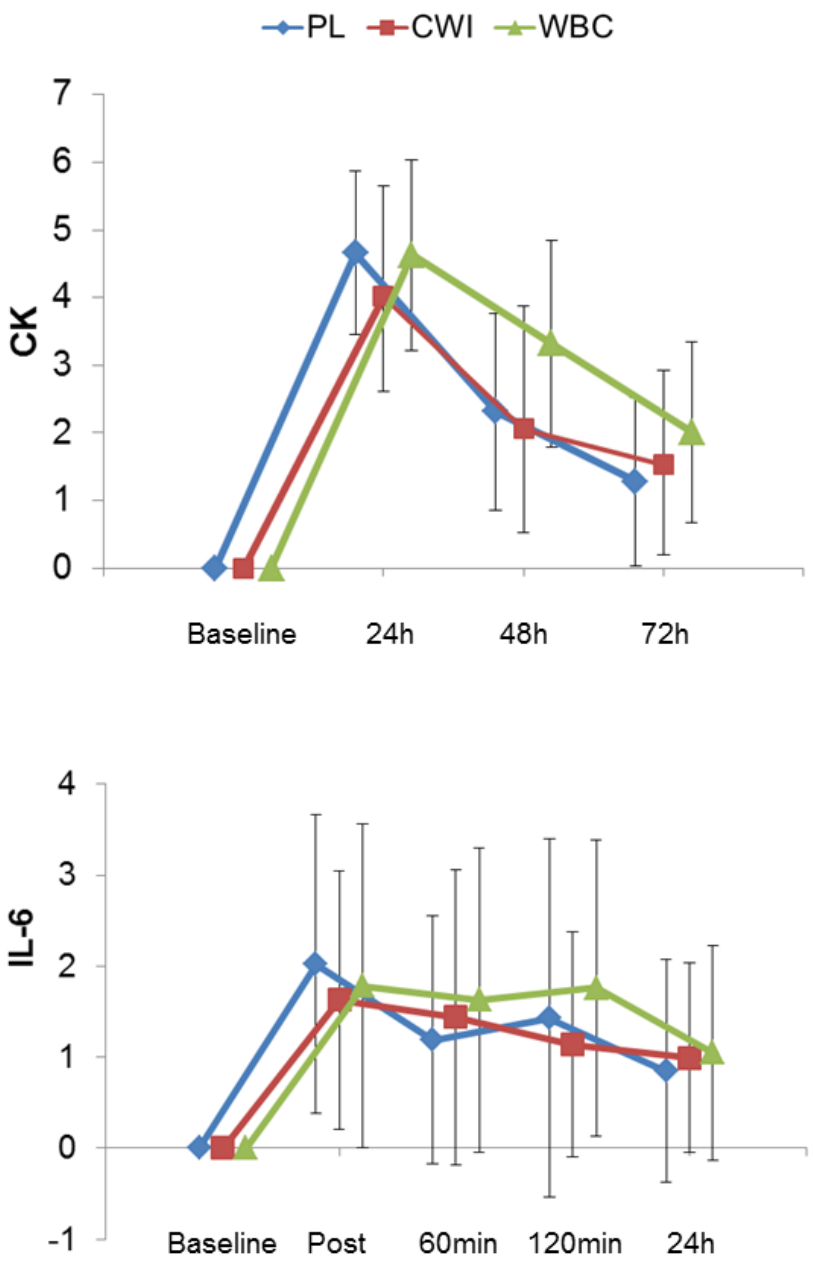

Figure 5.1. Factor change in CK and IL-6 with 90% CL error bars.

5.3.7.3. CRP

At baseline, CRP values were 1567.1 ± 2861, 1126.4 ± 1071.9 and 358.4 ± 220.8 µg/ml for placebo, CWI and WBC respectively. From baseline to 24 h, change over time analyses revealed a very likely small (2.43; x/÷1.54) and possibly likely small (1.29; x/÷1.26) increase for PL and CWI respectively whilst WBC demonstrated a

very likely moderate increase (1.95; x/÷1.51). At 48 h, both PL and CWI revealed a trivial change, whilst WBC demonstrated a likely moderate increase (2.06; x/÷1.65). At 72 h PL and CWI demonstrated an unclear effect whilst there was a possible moderate increase (1.51; x/÷1.69) for WBC. From baseline to 24 h CWI values demonstrated a likely large decrease compared to placebo (0.53; x/÷1.59), whilst comparisons at 48 and 72 h were unclear. WBC demonstrated an unclear effect compared to placebo at 24 h, but a possible moderate increase at 48 (1.53; x/÷1.85) and 72 h (1.62; x/÷2.00). WBC demonstrated a likely moderate increase compared to CWI at all time points (1.52; x/÷1.56, 2.04; x/÷1.88 and 1.89; x/÷1.86 at 24, 48 and 72 h respectively).

5.3.7.4. TNF-α

At baseline, TNF-α values were 38.7 ± 28.6, 35.7 ± 24.6 and 32.1 ± 34.7 pg/ml for placebo, CWI and WBC respectively. The time course of response differed amongst groups; peak values were recorded at 120 min, 72 h and 48 h post for placebo, CWI and WBC respectively. Change over time analyses revealed unclear or trivial effects for PL at all time points. CWI showed a small increase immediately post (1.39; x/÷1.92), an unclear, and then trivial change at 60 and 120 min. There was a likely moderate decrease for CWI at 24 h (0.39; x/÷2.71), an unclear change at 48 h and a likely moderate increase (1.90; x/÷1.84) at 72 h. Changes for WBC were trivial or unclear at all time points with the exception of a possible small increase (1.60; x/÷1.99) immediately post and a likely small increase (1.85; x/÷1.91) at 48 h. CWI demonstrated a likely moderate increase (2.23; x/÷3.59) compared to placebo immediately post, and a possibly small (1.48; x/÷3.62), and likely moderate (2.43; x/÷2.15) increase compared to placebo at 48 and 72 h respectively. Compared to placebo, WBC demonstrated a likely moderate increase (2.56; x/÷3.640) immediately post, a possibly small increase (1.52; x/÷2.73) at 60 min post and a likely large increase (3.94; x/÷3.51) at 48 h. Compared to CWI, WBC demonstrated

a possibly trivial increase (1.15; x/÷2.40) immediately post, a possibly small increase (1.41; x/÷3.55) at 60 min, and a likely moderate increase at 120 min (2.59; x/÷5.04), 24 h (2.86; x/÷4.17) and 48 h (2.66; x/÷2.46). All other group comparisons were unclear.

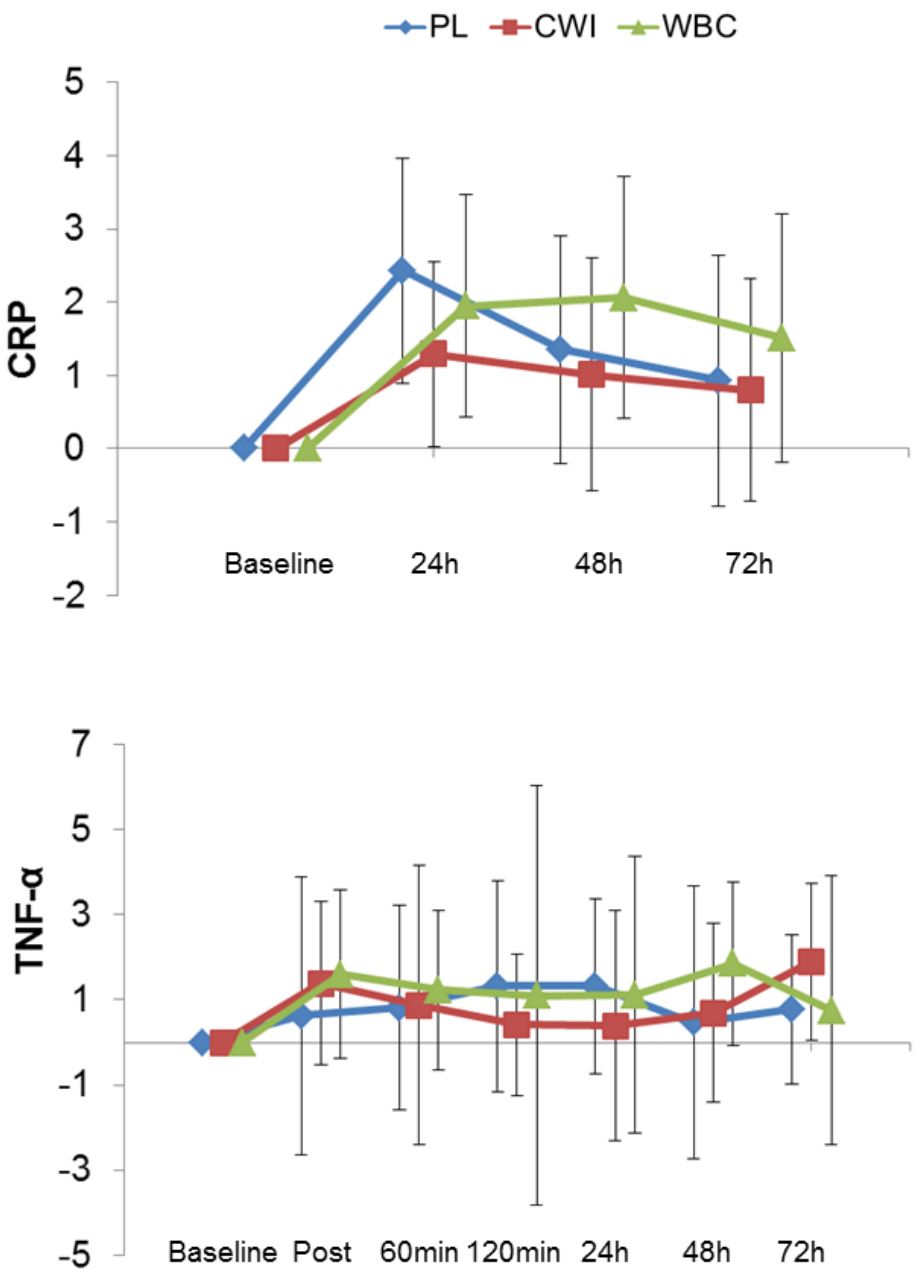

Figure 5.2. Factor change in CRP and TNF-α with 90% CL error bars.

Where there are large differences in baseline values between groups, this is attributed to one or two individuals who had values substantially greater than the normal range. However, as data was covariated using baseline values and results were analysed as the difference between groups in change over time, these participants were not removed from the analysis.

5.4. Discussion

The present study examined the effectiveness of a single bout of CWI or WBC, or a placebo intervention on markers of recovery in resistance trained males following a high volume, heavy load lower body resistance training session. The training session resulted in perturbations of muscle function, increases in perceptions of soreness and stress response symptoms and increases in blood borne markers of damage and inflammation. Overall, the results demonstrated little evidence to suggest that either cryotherapy intervention was more effective than a placebo at limiting decrements in muscle function, perturbations in perceptual responses or increases in inflammatory markers. Similarly, the majority of comparisons between CWI and WBC showed trivial or unclear results, although there was some evidence to suggest that WBC is more effective than CWI at attenuating detrimental increases in perceptual responses 24 h post exercise, and that CWI may have greater potential for reducing inflammation compared to WBC.

For both cryotherapy interventions, DALDA scores were unclear, trivial or increased compared to the placebo intervention at all time points. However, from baseline to 24 h, WBC demonstrated a likely beneficial effect compared to CWI. These findings are supported by the findings from the first study which showed that whilst neither CWI nor WBC offered any perceptual benefit over a placebo intervention, WBC was superior to CWI when monitoring recovery following a marathon. These findings lend further support to the suggestion that many of the therapeutic effects attributed

to cryotherapy interventions may in fact be ascribed, at least in part, to a placebo effect (Broatch et al., 2014). In terms of muscle soreness, CWI showed a trivial effect compared to the placebo intervention at all time points, but from baseline to 24 h WBC was likely beneficial compared to both the placebo and CWI condition.

Maximal isometric strength at 90° decreased in all groups following completion of the resistance training session and remained diminished at 72 h. Group comparisons revealed trivial or unclear effects of WBC compared to placebo and CWI at all time point. For MVIC at 90°, CWI demonstrated moderate and small reductions compared to placebo at 24 and 48 h respectively. Similarly, in terms of peak force assessed via maximal isometric squats, despite all group comparisons being unclear at 24 and 72 h, CWI demonstrated a large performance reduction compared to moderate reduction in the placebo group at 48 h. These findings are in contrast to Vaile, Halson, Gill, & Dawson (2008) who reported smaller peak force performance decrements in the CWI group (-7.3%) compared to a passive recovery group (-15.7%) following a DOMS-inducing eccentric leg press protocol. Methodological differences may help to explain the opposing findings. In the present study, participants completed a higher volume training session (120 versus 70 repetitions) which may have resulted in greater muscle damage, evidenced by greater peak force decrements at 48 h (-29.8 vs -7.3% for CWI and -18.8 vs -15.7% for placebo/control). Secondly, the CWI intervention used in the study by Vaile and colleagues (2008) implemented a 14 min 15°C protocol whereas the present study utilised a 10 min 10°C protocol. This lends weight to the recommendation from Machado and colleagues (2016) that CWI at a temperature between 11 and 15°C for 11–15 min may provide the best results for both immediate and delayed effects. Further, as is becoming more important in cryotherapy literature (Broatch et al., 2014), the present study employed a placebo, rather than a control group which may strengthen the study design and provide greater ecological validity. RFD is

considered a more specific and sensitive indirect measure (Maffiuletti et al., 2016; Peñailillo et al., 2015) of muscle damage after exercise than MVIC. The RFD data from 100-200 ms largely mirrors the peak force data, suggesting that WBC was most beneficial at 48 h compared to CWI and placebo. However, unlike the peak force data, WBC demonstrated a reduced effect compared to the placebo at 24 h, so without further data the potential beneficial effects of WBC on peak force and RFD at specific time points should be interpreted cautiously.

For peak torque at $60°\cdot s^{-1}$ both cryotherapy interventions attenuated recovery compared to the placebo at 24 h. Further, CWI demonstrated a reduced recovery response compared to the placebo at 48 h. Comparisons between CWI and WBC were trivial or unclear at all time points, which is in contrast to the findings from the first study of the thesis which found that recovery of peak torque at $60°\cdot s^{-1}$ in WBC was reduced compared to CWI following a trail marathon. The selection of any outcome variable utilised to assess recovery after exercise should be specific to the exercise stress itself, and a time trial (although methodologically challenging) may have been more appropriate in the previous investigation. For this reason, peak torque values reported in the previous study may not accurately represent performance decrements following a prolonged endurance exercise stress.

Cryotherapy had a trivial or reduced impact on recovery of CMJ compared to the placebo at all time points. However, at 24 h WBC was less effective than CWI, and this finding is supported by Abaïdia et al. (2016) who reported that there was a very likely moderate effect in favour of CWI for CMJ recovery compared to WBC 72 h after exercise. A recent investigation from Hohenauer et al. (2018) demonstrated that there was no difference between CWI and PBC for functional recovery assessed via MVCs and vertical jumps. However, it is worth noting that neither study employed a placebo or control condition, so recommendations relating to the efficacy of cryotherapy should be interpreted with caution. Furthermore, differing

exercise stresses (dynamometry and repeated drop jumps respectively) make it difficult to directly compare findings to the present investigation. In terms of RSI derived from the drop jump data, both cryotherapy interventions demonstrated a reduced or unclear effect compared to the placebo intervention. However, in contrast to the jump height data, WBC was most likely beneficial compared to CWI at 24 h. This result may indicate the influence of a learning effect from baseline to the 24 h post testing session. Change over time analyses show that RSI values in the WBC group demonstrated a possibly beneficial effect at 24 h, whereas there was a decrease in both the placebo and CWI group at the same time point.

The finding that cryotherapy was ineffective at attenuating increases in CK following exercise is supported by Jakeman, Macrae and Eston (2009) who reported that despite peaking 24 h post plyometric exercise, there were no differences in CK between the CWI and control group. Similarly, the results are supported by the first study of this thesis, which demonstrated that following completion of a trail marathon WBC was less effective than CWI at tempering increases in CK.

A key mechanism purported to support the use of cryotherapy as a recovery intervention is that it can modulate blood flow and cell metabolism (Mawhinney et al., 2017), resulting in an attenuated inflammatory response (Tipton et al., 2017). The results from the present study do not support this premise, with cryotherapy largely trivial or less effective compared to the placebo intervention. In line with previous research, IL-6 peaked immediately post exercise (Roberts et al., 2014), however, all group comparisons were trivial, suggesting no meaningful difference between interventions. Given that IL-6 is an acute phase inflammatory marker that often peaks immediately post exercise, it is possible that cryotherapy applied following exercise can have little impact on circulating levels. This is in line with Selfe et al. (2014) who reported that a single WBC exposure, irrespective of duration (1, 2 or 3 min), did not significantly alter circulating IL-6 following a game of rugby league. The

results from the CRP and TNF-α analyses demonstrated that CWI may offer slight benefits compared to WBC, but that there was no benefit of cryotherapy compared to the placebo intervention. These findings are supported by White, Rhind and Wells (2014) who reported that CWI (10°C x 10 min) following high intensity exercise does not reduce plasma markers of inflammation, and that prolonged CWI (10°C x 30min) can actually exacerbate the inflammatory response. Similarly, previous research has suggested that 'severe cold' immersion protocols (5-10°C) can negatively impact upon recovery, by eliciting a cold related stress response (Machado et al., 2016). This in turn could escalate the inflammatory cascade response, increase perceptions of soreness and ultimately impact on functional recovery (Machado et al., 2016), which was evidenced in the first study of this thesis.

Potential limitations of the current study should also be addressed. The WBC treatment temperature utilised in the present study was considerably warmer than that normally reported in the literature (-85° versus -110° to -140°C), therefore, although the findings add to the current body of literature, the findings cannot be generalised to colder exposure temperatures. Secondly, there were large variations in baseline values for a number of outcome measures. For the blood markers, all results are reported as factor change over time and baseline values were used as a covariate. All participants avoided strenuous exercise for a minimum of 48 h before the baseline session, and it is likely that large variations are present in physically active populations. The pattern and magnitude of change was not largely different in participants who had large baseline values, compared to those with lower values. Participants were matched into groups based on lean mass and predicted 1RM, and as a result there were differences in absolute strength between groups. However, all functional outcomes were reported as percentage change to minimise the potential confounding effect of absolute raw values. Lastly, there was no direct measure of

expectance effect or treatment belief in the present study, which could have strengthened the findings.

5.5. Conclusion

The overall aim of the thesis was to add to the current body of knowledge relating to the effects of acute and habitual cryotherapy exposure on recovery and adaptation following strenuous exercise, using placebo-controlled study designs. In terms of the specific thesis aims, this study addressed aim 1 (to establish whether cryotherapy is any more effective than a placebo intervention for recovery following an acute bout of strenuous exercise), 2 (to determine whether WBC is any more effective than CWI) and 3 (to establish whether exercise mode influences the impact of cryotherapy on recovery). When comparing the efficacy of the different cryotherapy modalities on recovery following resistance training, although WBC demonstrated some beneficial effects compared to CWI, comparisons were largely unclear, trivial or favoured the CWI condition. These findings, in addition to those from the first study of the thesis, and Abaïdia et al. (2016) add more weight to the argument that WBC offers few additional benefits over CWI for recovery following strenuous exercise. Similarly, in terms of investigating the contribution of a potential placebo effect associated with cryotherapy, the majority of group comparisons revealed unclear, trivial or unfavourable effects of cryotherapy compared to the placebo intervention, contradicting much of the previous literature. Again, this echoes the findings from the first study of the thesis and highlights the need for future cryotherapy studies to implement an effective placebo controlled design.

Although the findings from the first two studies of this thesis offer little evidence to support the use of cryotherapy post exercise, it remains a popular intervention (Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015). Additionally, given that many of the proposed benefits of cryotherapy treatment are contingent on its ability

to attenuate the inflammatory response, it is important to understand whether interrupting the inflammatory cascade may impact upon the normal cycle of muscle damage and repair. As such there is scope to investigate the impact of habitual cryotherapy exposure on potential (mal)adaptations to an ecologically valid training programme.

Chapter 6

The impact of habitual post exercise CWI exposure on adaptations to resistance training.

6.1. Introduction

The first two studies of this thesis examined the efficacy of different cryotherapy modalities on markers of recovery following strenuous endurance, and resistance exercise respectively. The results from those studies demonstrated that whilst there may be some perceptual benefits of cryotherapy, when compared to a placebo intervention, there was little evidence to suggest that either CWI or WBC attenuated inflammation or provided enhanced functional recovery benefits. However, cryotherapy is still widely used as a recovery intervention following strenuous exercise (Hohenauer et al., 2015). The majority of literature relating to acute cryotherapy exposure, including the first two studies of this thesis, focuses on trying to attenuate the impact of strenuous exercise on functional or perceptual decrements in an attempt to enhance recovery. However, as previously discussed, although strenuous exercise leads to a number of degradative pathways related to muscle breakdown and inflammation (Brancaccio et al., 2010; Proske & Allen, 2005), these processes form a crucial part of the breakdown and regeneration cycle required for adaptation (Fröhlich et al., 2014; Niess & Simon, 2007; Paulsen et al., 2012; Tipton et al., 2017; Vieira et al., 2016). Given that many of the underpinning mechanisms purportedly responsible for the efficacy of cryotherapy treatment rely on interrupting or diminishing the processes involved in this cycle, it follows that repeated exposure could result in maladaptive responses to specific training stimuli (Fröhlich et al., 2014; Yamane et al., 2006). Put more simply, neutrophilia and/or an influx of other cytokines leads to the removal of debris from the cells and stimulation of satellite cells, leading to regeneration and repair (Kendall & Eston, 2002). If cryotherapy exposure modulates blood flow (Mawhinney et al., 2017) and attenuates the inflammatory response (Tipton et al., 2017; Vieira et al., 2016), this may be beneficial in the short term, or tournament situations where repeated high level performance is vital, but in the long term it could dampen the anticipated

training response. With few longitudinal studies examining long term effects of repeated cryotherapy exposure, it is pertinent to examine the influence of habitual use on adaptations to training.

Given that strength training underpins a large proportion of programming in a variety of sports (Young, 2006), it was determined that a resistance based exercise training programme would be used in the present study to maximise the impact of the findings. A recent review from Broatch, Petersen and Bishop (2018) states that, to date, the available evidence suggests either no effect, or negative effects, of post exercise CWI on adaptations to resistance training. Yamane and colleagues (2006) utilised a 4 week hand grip exercise programme to examine the effect of repeated unilateral CWI (20 min at 10°C) on improvements in muscle performance. Their results showed that maximal muscle strength, assessed via hand grip dynamometry, increased in both limbs, although there was no difference between cooled and control limbs. Later, Fröhlich et al. (2014) utilised a 5 week leg curl strength training programme to examine the influence of repeated unilateral CWI (3 x 4 min at 12°C) and found that 1RM and 12RM improved to a greater extent in the control condition. Both of these studies are limited in that cooling was applied unilaterally and this may have impacted on comparisons between cooled vs control limbs in the same individuals (Peake et al., 2017). Similarly, the exercise protocols used in both studies lacked ecological validity and therefore it is difficult to extrapolate their findings to an applied setting. More recently, Roberts et al. (2015) employed a more ecologically valid training programme to examine the effect of repeated CWI (10 min at 10°C) or active recovery (ACT) on changes in muscle mass and strength after 12 weeks of lower body resistance training. The authors reported that strength and muscle mass increased more in the ACT group than CWI. In addition, the authors conducted muscle biopsies to understand the influence of CWI and ACT on hypertrophy signalling pathways and satellite cell activity in skeletal muscle after

acute strength exercise. The findings from this portion of the investigation demonstrated that CWI blunted the activation of key proteins and satellite cells ($NCAM^+$ and $Pax7^+$) for up to 2 days after exercise, which may explain the improved strength and mass outcomes for participants in the ACT group. The authors postulated that regular suppression of satellite cell activity could lead to a cumulative detrimental response over time. Taking into account the previously mentioned investigations, examining the impact of a bilateral cooling intervention on functional and perceptual responses to an ecologically valid resistance training programme of <12 weeks would offer new knowledge. The use of 2 distinct training blocks may also more closely replicate the type of training cycles employed by athletes (Bartolomei et al., 2014), and could indicate whether any detrimental impact of cryotherapy is evident after as little as 4 or 8 weeks of exposure. Further, previous studies have tended to focus on selective elements of adaptation (such as functional *or* molecular markers) but in line with the previous studies of this thesis, the current investigation will take a holistic approach and address a range of functional and perceptual markers, as well as including more novel elements such as ultrasound and markers of bone and collagen turnover.

Although to date no research has investigated the long-term impact of WBC on adaptation, and the previous studies in this thesis have compared CWI and WBC, CWI remains the more widely used and accessible modality of the two (Holmes & Willoughby, 2016). Previous research has shown that CWI has a greater capacity to attenuate blood flow than WBC (Mawhinney et al., 2017) which may modulate the natural acute inflammatory response. This is supported by the findings from the previous study in this thesis, which demonstrated a greater impact of CWI on inflammatory markers compared to WBC, although in the context of habitual use, a dampened inflammatory cascade could in fact be detrimental for training adaptations.

Therefore the aim of this study was to investigate the influence of repeated CWI or a placebo intervention on perceptual, functional and structural adaptations to a progressive 2 x 4 week high volume, heavy load lower body resistance training programme. In addition, by including ultrasound and markers of bone and collagen turnover, the study aimed to offer insight into potential mechanisms relating to CWI exposure and (mal)adaptation to resistance training. It was hypothesised that repeated CWI exposure would attenuate physiological and functional adaptations to the strength and power training programme.

6.2. Methods

6.2.1. Participants

Thirteen healthy males volunteered to participate in the study (age 26 ± 6 years, height 1.8 ± 0.1m and mass 83.6 ± 15.7 kg). Participants were recruited using targeted social media, and were familiar with strength training (minimum 1 years' experience). Participants were able to maintain their normal sporting habits/routine, but were asked to refrain from any strength or power based training other than the prescribed sessions for the duration of the study. For the duration of the study period, participants were asked not to consume any nutritional (e.g. creatine/protein; antioxidants) supplements or non-steroidal anti-inflammatory drugs, and not to take part in any other recovery interventions (e.g. compression garments, massage, electrostimulation).

6.2.2. Study design

At a familiarisation session participants had their initial DXA scan, completed assessments of their RMs for all the exercises in the strength phase of the programme, and were then familiarised with all DV's listed below. Total lean mass in kg (determined from DXA scan) and back squat 4RM were used to match participants into either the CWI or placebo group. One week following the

familiarisation session, baseline values were taken for ultrasound and all functional DV's, which were repeated at mid-point and post. The following week, participants began the training programme. This consisted of 2 x 4-week blocks separated by one rest week in which mid-point data was collected. Participants trained 2 days/week, with at least 24 h between sessions (usually Monday and Wednesday or Tuesday and Thursday). On the morning of-, 24 and 48 h post the first and last training session of each training block (training sessions 1, 8, 9 and 16) participants completed muscle soreness and perceived recovery questionnaires. Blood samples were taken immediately before the first sessions of block 1 and 2, and the last session of block 2, which were used as baseline, mid- and post-testing values. Further, the morning after the 2nd training session of the first and last week of each block, participants completed a DALDA questionnaire. Lastly, sleep efficiency was recorded using actigraphy and self-report sleep questionnaires during the first and last week of each training block. Repeat DXA scans and measurements of all DV's (with the exception of blood markers and questionnaires) were recorded in the week separating the strength and power blocks of training (mid-point), as well as in the week following completion of the training programme (post). An overview of the study outline is provided in Table 6.1.

Table 6.1. Overview of study design

	Strength block (sessions 1-8)						Power block (sessions 9-16)					
	F	**B**	1	2	3	4	**M**	5	6	7	8	**P**
DXA	✓						✓					✓
RMs	✓											
US		✓					✓					✓
Function	✓	✓					✓					✓
Blood			✓					✓			✓	
DOMS			✓			✓		✓			✓	
P/REC			✓			✓		✓			✓	
DALDA			✓			✓		✓			✓	
Sleep			✓			✓		✓			✓	
PL/CWI			✓	✓	✓	✓		✓	✓	✓	✓	

F, Familiarisation; B, Baseline; M, Mid; P, Post; DXA, DXA scan; RMs, repetition maximums; US, Ultrasound; Function, muscle function (dynamometry, drop jumps, countermovement jumps and isometric squat); Blood, blood sample; DOMS, perceptions of soreness; P/REC, perceptions of recovery; DALDA, Daily Analysis of the Lifestyle Demands of Athletes questionnaire; Sleep, sleep efficiency measured with actigraphy; CWI/PL, recovery intervention.

6.2.3. Calculation of repetition maximums (RMs)

Please refer to section 3.3.4.

6.2.4. Training programme

The training programme was split into two phases each lasting 4 weeks: phase 1 was strength focused and phase 2 was power training. The strength training programme comprised 5 exercises (jump shrugs (4 x 2), ¼ squat (4 x 4), split squat (4 x 4 per leg), barbell hip thrusts (3 x 6) and Romanian deadlift (3 x 6)). All exercises were performed at RM for the number of reps in each set, e.g. split squat at 4RM.

The power training programme also comprised 5 exercises (¼ squat, jump shrugs, box jumps, squat jumps and drop jumps). All exercises were performed for 5 sets of 3 repetitions, with the exception of the ¼ squats which were completed in 4 sets of 2. Drop jumps were performed using a 30 cm box.

Loads for each exercise were progressively increased for each participant throughout the programme. In order to be included in the final analysis, participants were required to attend 15 out of the 16 training sessions. This type of training programme has previously been used successfully to investigate adaptations to training (Cormie, McGuigan, & Newton, 2011; Cormie, McGuigan, & Newton, 2010). The specific training programme implemented in the present study was developed in conjunction with an accredited strength and conditioning expert. Session RPE (sRPE) was collected after the first and last session of each training block and the results demonstrated that there were no clear differences between groups at any time point.

6.2.5. Dependent variables

6.2.5.1. Muscle soreness

Participants indicated their perceived levels of muscle soreness of the lower limbs during a body weight squat (approx. knee angle of 90°) using a Likert scale as described in section 3.3.2. This measure was recorded the morning of-, 24 and 48 h following the first and last exercise session of each training block (Table 6.1). Participants were emailed unique links to an online version of the scale so it could be completed on a computer or smart phone. Participants completed the questionnaire within 60 minutes of waking on the relevant days.

6.2.5.2. Perceived recovery

Participants were asked to record their perceived level of recovery at the same time points as muscle soreness as described above. Participants were asked to rate how recovered they felt on a 0 (very poorly recovered/extremely tired) to 10 (very well recovered/highly energetic) scale (Appendix 7). As with muscle soreness, participants were emailed individual links to the scale and it could be completed online. Participants completed the questionnaire within 60 minutes of waking on the relevant days.

6.2.5.3. DALDA

Participants were required to complete a DALDA questionnaire the morning after the 2nd training session of the first and last week of each training block. Each participant was emailed a unique link in order to complete the questionnaire online so they did not need to attend the laboratory. Further detail relating to the DALDA questionnaire is included in section 3.3.3.

6.2.5.4. Sleep efficiency

For the first and last week of each training block, participants were required to wear an actigraph monitor (GeneActiv, Activinsights, Cambridgeshire, UK), which is a wristwatch like device capable of continuously monitoring body movement over a pre-determined period of time; participants' sleep data was recorded over a 7 night period (Saturday to Saturday) in 60 second epochs (Halson, 2014b). Sleep actigraphy was used in conjunction with self-reported sleep diaries (Appendix 8) to determine when participants were asleep and awake. Participants were asked to record the time at which they attempted to sleep after getting into bed each night, as well as the time they woke up each morning. All time was scored as awake unless the diary showed participants were lying down attempting to sleep and the activity monitor displayed a sufficiently low activity count for that period (Halson, 2014b).

Sleep data was analysed in Microsoft Excel using the GeneActive sleep macro, and sleep efficiency was calculated as a percentage for each recorded night of sleep $\left\{\frac{Sleep\ time\ (min)}{Elapsed\ sleep\ time\ (min)} \times 100\right\}$ then averaged over each 7 night period to give weekly mean values. Sleep diaries were used to verify the data from the actigraph watches. Actigraphy has been used previously in conjunction with self-report sleep diaries to monitor sleep quality and quantity in athletes (Hausswirth et al., 2014; Robey et al., 2014).

6.2.5.5. Dual x-ray absorptiometry (DXA) scan

Body composition of all subjects was assessed using a DXA scanner (fan beam, Lunar Prodigy 4, GE Medical Systems, Lunar, Madison, WI, USA) during the familiarisation session in order to match participants into treatment groups. Lean mass was also measured at baseline, mid and post. Scans were conducted in accordance with the procedures detailed in section 3.3.1.

6.2.5.6. Ultrasound

Ultrasound images of the vastus lateralis (VL) of the right leg were taken at rest. Participants lay in a supine position, with their knees supported at the end of the bed, but their lower legs hanging over the end so that measurements were standardised. Portable ultrasound equipment (MyLab™30Gold Cardiovascular, ESAOTE, Bracco UK, Buckinghamshire, UK), was used to acquire longitudinal images of the VL. The probe was placed at 50% of thigh length, defined as the distance from the greater trochanter to the popliteal crease. Ultrasound gel was used to enhance image quality and minimise pressure over the skin, in order to avoid tissue compression and deformation of muscle architecture variables. Using Image-J software, muscle fibre pennation angle was calculated as the acute angle formed between the deep aponeurosis and a muscle fascicle (e Lima, Carneiro, Alves, Peixinho, & de Oliveira, 2015). A previous reliability study demonstrated that

the typical error of measurement for this variable ranges from 0.08 to 1.66 degrees, with intraclass correlation coefficients and CV's from 0.78% to 0.99% and 2.15% to 9.68%, respectively (e Lima et al., 2015). In the present study, data collection and interpretation was conducted by a trained clinical physiologist to ensure reliability.

6.2.5.7. Peak torque and isometric contractions

Please refer to section 3.3.5.

6.2.5.8. Reactive strength index (RSI)

Please refer to section 3.3.7.

6.2.5.9. Unloaded and loaded countermovement jumps

Leg extensor strength and power assessment was conducted using unloaded and loaded counter movement jumps. Participants were required to complete 6 (3 x 2) maximal CMJs at different loads. In order to maintain the same posture and technique across loads, the warm up and unloaded jumps were conducted using a wooden broom handle (~100 g) in place of a barbell. Following 3 warm-up jumps, participants performed 3 sets of 2 maximal CMJs with increasing loads; unloaded (broom handle), 20 kg and 50 kg. Loaded and unloaded jumps have been used previously to assess the effectiveness of resistance and plyometric training in trained individuals (Marques, Van Den Tillaar, Vescovi, & González-Badillo, 2008). Participants were provided with 30 seconds rest between repetitions, and 2 minutes rest between each set. Each CMJ followed the same procedure; from a relaxed standing position participants made a countermovement to a squat position (self-selected depth) before jumping vertically for maximum height. Each jump was performed in a continuous movement with the hands remaining on the bar throughout. All jumps were performed on a portable force plate (Kistler, Switzerland) at a sampling frequency of 1000 Hz interfaced with a laptop. Any efforts that

deviated from the prescribed technique were deemed void and repeated. Raw data was analysed in accordance with Chavda et al. (2017), with jump height values from each testing session used for statistical analysis.

6.2.5.10. Peak force and rate of force development

Peak force and RFD parameters were recorded in accordance with the procedure outlined in section 3.3.6.

6.2.5.11. Blood

Venous whole blood samples were collected and processed as described in section 3.3.9. Samples were analysed for PINP and PIIINP. PINP is derived from collagen type I and whilst it is the most common collagen type found in mineralised bone, it is also present in soft connective tissues within the extracellular matrix (Jensen et al., 2002). PINP can be found in the circulation and its concentration reflects the synthesis rate of collagen type I, and is considered the most sensitive marker of bone formation (Banfi, Lombardi, Colombini, & Lippi, 2010). Similarly, PIIINP is the amino terminal peptide of type III procollagen, released during the synthesis and deposition of type III collagen. PIIINP in the circulation can be derived from synthesis of new type III collagen or from the degradation of existing type III collagen fibrils in muscle (Berry et al., 2013).

6.2.5.11.1. PINP

Plasma PINP concentration was measured using a standard QS ELISA (Aviva Systems Biology, San Diego, USA). The reported assay ranges are 3.12 – 200 ng/mL, the minimum detection concentration (MDC) is < 1.6 ng/mL with an intra-and inter-assay CV of < 6.5% and < 8.7% respectively.

6.2.5.11.2. PIIINP

Plasma PINP concentration was measured using a standard QS-ELISA (Aviva Systems Biology, San Diego, USA). The reported assay ranges are 0.3-20 ng/mL, the MDC is < 0.3 ng/mL with intra-and inter-assay CV of < 5.1% and < 8.0% respectively.

6.2.6. Nutritional supplementation

In an attempt to minimise potential variation in training responses, participants were provided with standardised nutritional supplements. All participants were given individual servings of a whey protein isolate (The Protein Works, Cheshire, UK) equivalent to 0.4g·kg^{-1} body mass to consume ~1h before, and within 15 min following completion of each training session. They were also given two small recovery bars (Protein Grazers, The Protein Works, Cheshire, UK) each containing 7.4 g protein and 14.2 g carbohydrate to consume 2 hours after each training session. The nutritional supplementation strategy utilised in this study is similar to that used by Roberts et al. (2015), however, rather than providing a standardised serving of whey protein, doses were individualised to take into account large variations in body mass. By using 2 x 0.4 g·kg^{-1} doses on training days, it was hoped that all participants would reach the minimum 1.4-2 g·kg^{-1} protein recommended for physically active individuals (Campbell et al., 2007). From 1 h before, until 2 h post each training session, participants were only allowed to consume the supplements provided, and water. The participants were instructed to avoid consuming any additional dietary supplements and to follow their habitual diet for the duration of the study. Dietary intake was monitored using 7-day food diaries, completed during the first and last week of training. Analysis revealed that, when adjusted for body mass, there were no clear differences in total energy (Kcal) or

macronutrient (carbohydrate, protein and fat) intake between groups at either time point.

6.2.7. Interventions

6.2.7.1. Placebo

The importance of implementing an effective placebo has become increasingly evident as the studies in this thesis have progressed. As already stated, all participants in the study were provided with protein supplements before and after each training session, as well as protein snacks to consume 2 hours post exercise. However, participants in the placebo group were informed that their protein supplements contained additional leucine. Leucine is an essential branched chain amino acid utilised in the synthesis of proteins, and a key nutrient 'trigger' for muscle anabolism (Norton & Layman, 2006).

6.2.7.2. Cold water immersion (CWI)

Following each training session, participants in the CWI group completed their allocated intervention. The protocol is described in detail in section 3.4.2.

6.2.8. Statistical analysis

The majority of statistical analyses were conducted in accordance with the procedures previously described in section 3.6. However, in this study, perceptions of soreness and recovery were collected at multiple time points *and* over multiple weeks. Therefore, when analysing these variables, delta scores were calculated for baseline to 24 h and baseline to 48 h and then compared from week 1 to week 4, and week 5 to week 8 in order to investigate group differences. An SWC of 1 was used in line with the previous studies.

6.3. Results

Analysis of pooled data revealed that the training programme resulted in clear increases in back squat 4RM, although there was no clear change in total lean mass. There were clear increases in muscle fibre pennation angle, RSI, isometric peak force, and RFD at both 50-100 ms and 100-200 ms. Whilst peak torque at 60°·s^{-1} did not change, there was an improvement in peak torque at 180°·s^{-1}. Unloaded CMJ height showed a small decrease over time, whilst there was no change in loaded CMJ outcomes. In terms of perceptual responses, there was an increase in stress reaction symptoms during the strength training block and then a decrease during the power training block. This was reflected in the sleep efficiency data which demonstrated a decrease, and then an unclear change across the two training blocks. Across the first training block, DOMS decreased and perception of recovery increased at both 24 and 48 h post exercise. In the second block, DOMS increased at 24 h but there was an unclear change at 48 h. Changes in perceptions of soreness were trivial across the second training block. The outcomes for group specific changes over time as well as group comparisons for all parameters can be seen in tables 6.2, 6.3, 6.4 and 6.5.

6.3.1. Muscle soreness

Changes in perceptions of soreness were assessed by examining the magnitude of change of score differences from pre-24 h and pre-48 h from week 1 to week 4 and week 5 to week 8. For changes between week 1 and 4, the placebo group demonstrated a moderate, and large decrease, and changes in the CWI group were unclear at both time points. As a result, group comparisons showed small effects in favour of the placebo group at both time points (24 and 48 h) from week 1 to 4. For weeks 5 to 8, both groups demonstrated a small increase from pre- to 24 h, with

trivial or unclear changes for pre- to 48 h. Group comparisons were trivial and unclear for 24 and 48 h respectively for week 5 to 8.

6.3.2. Perceived recovery

Perceived recovery scores were analysed in the same manner as perceptions of soreness as described above. However, in contrast to the soreness data, an increase in scores indicates an improved perception of recovery. Change over time analyses revealed trivial, and very large increases for pre-24 h and pre-48 h respectively for PL from week 1 to 4. The CWI group showed large increases at both time points. Group comparisons were trivial and unclear. For week 5 to 8, PL showed unclear and trivial changes, whilst CWI demonstrated small, and trivial decreases at 24 and 48 h respectively. These findings resulted in a small effect in favour of PL at 24 h and a trivial comparison at 48 h from week 5-8.

6.3.3. DALDA

At baseline, DALDA scores marked worse than normal were 3 ± 3 for both the PL and CWI groups. There were small, and trivial increases from week 1 to 4 for PL and CWI respectively. From week 5 to 8 there was an unclear change in PL and a small decrease in CWI. Group comparisons were unclear for both time points.

6.3.4. Sleep efficiency

At baseline, sleep efficiency values were 76.25 ± 16.37 and 63.92 ± 16.32% for the PL and CWI groups respectively. PL demonstrated a small, and then trivial decrease for weeks 1 to 4 and 5 to 8 respectively, whilst changes for CWI were unclear. Group comparisons were unclear at both time points.

Table 6.2. Change over time and group comparisons for perceptual markers

		Changes over Time		Effects
		Mean; ± CL		Mean[a]; ± CL[b]
		Qualitative Outcome		Qualitative Outcome
		Placebo	CWI	PL/CWI
Muscle Soreness	Pre-24h	-2.0; ± 2.5	-0.8; ± 2.4	1.2; ± 3.2
	Wk 1-4 Δ	Moderate ↓**	Unclear	Small ↑*
	Pre-48h	-4.0; ± 1.7	-2.3; ± 3.3	1.7; ± 3.5
	Wk 1-4 Δ	Large ↓***	Unclear	Small ↑*
	Pre-24h	0.5; ± 1.4	1.0; ± 2.5	0.5; ± 2.7
	Wk 5-8 Δ	Small ↑*	Small ↑*	↔*
	Pre-48h	-0.4; ± 1.3	-2.2; ± 3.2	-1.8; ± 3.3
	Wk 5-8 Δ	↔**	Unclear	Unclear
Perceived Recovery	Pre-24h	0.5; ± 1.0	4.5; ± 4.7	4.0; ± 4.8
	Wk 1-4 Δ	↔**	Large ↑**	Unclear
	Pre-48h	4.2; ± 2.9	3.6; ± 2.1	-0.6; ± 3.3
	Wk 1-4 Δ	Very large ↑***	Large ↑***	Trivial ↓*
	Pre-24h	0.7; ± 2.8	-0.9; ± 1.9	-1.6; ± 3.1
	Wk 5-8 Δ	Unclear	Small ↓*	Small↓*
	Pre-48h	0.4; ± 1.4	-0.1; ± 2.6	-0.5; ± 2.8
	Wk 5-8 Δ	↔*	Trivial ↓*	↔*
DALDA	Wk 1-4	1.5; ± 2.1	0.3; ± 4.2	-1.2; ± 3.8
		Small ↑*	Trivial ↑*	Unclear
	Wk 5-8	-1.0; ± 4.8	-4.5; ± 5.0	-3.7; ± 5.4
		Unclear	Small ↓**	Unclear
Sleep Efficiency	Wk1-4	-5.4; ± 18.0	2.0; ± 12.2	7.4; ± 19.0
		Small ↓*	Unclear	Unclear
	Wk 5-8	-1.7; ± 12.0	6.3; ± 11.9	8.0; ± 5.2
		Trivial ↓*	Unclear	Unclear

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease, and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

6.3.5. Lean mass

At baseline, lean mass values were 70.12 ± 8.8 and 55.37 ± 6.5 kg for PL and CWI respectively. Changes in lean mass were trivial for both groups at both time points, leading to trivial group comparisons (Table 6.5).

6.3.6. Muscle fibre pennation angle

At baseline, pennation angles were 20.7 ± 2.0 and 18.1 ± 2.5° for PL and CWI respectively. Pennation angle increased from baseline in both groups at both time points. From baseline to mid, there was an unclear effect, but from baseline to post, PL demonstrated greater increases than CWI. Figure 6.1 shows examples of muscle scan images after analysis of pennation angle using Image-J.

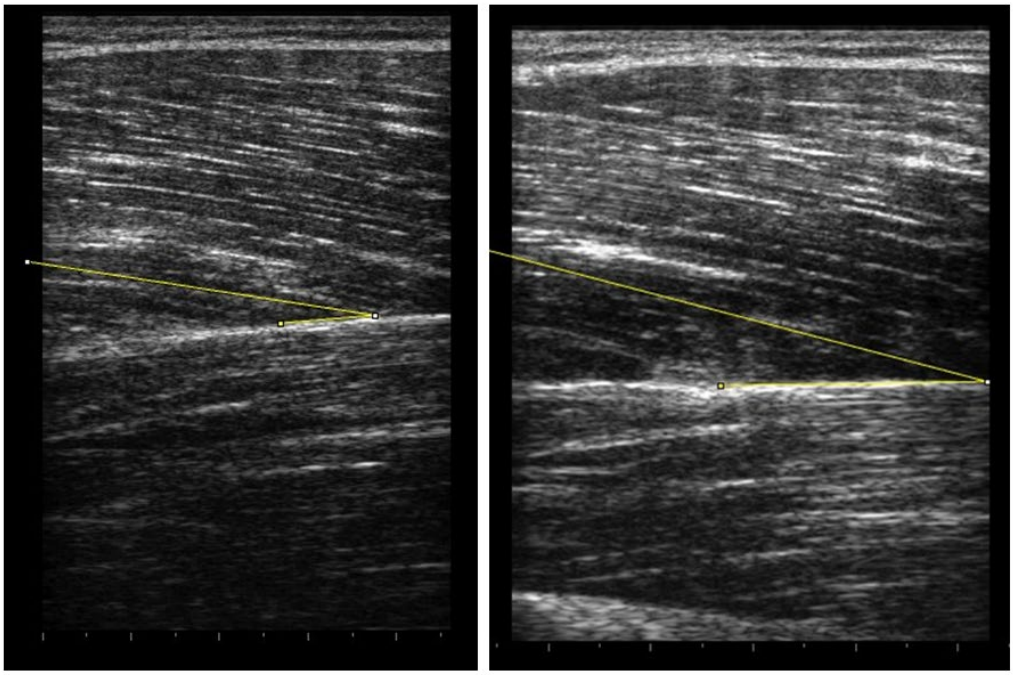

Figure 6.1. Ultrasound images of the VL showing alterations in fibre pennation angle from baseline *(left)* to post *(right)*.

6.3.7. Maximal voluntary isometric contractions and peak torque

6.3.7.1. MVIC 90°

At baseline MVIC values at 90° were 281.8 ± 56.5 and 237.9 ± 57.1 Nm for PL and CWI respectively. Changes over time revealed trivial changes for both groups at mid and post, resulting in unclear group comparisons at both time points.

6.3.7.2. MVIC 30°

At baseline, MVIC values at 30° were 140.2 ± 29.4 and 113.9 ± 25.9 Nm for PL and CWI respectively. From baseline to post, the PL group demonstrated a small decrease whilst the CWI group exhibited a small increase. Therefore, the group comparison from baseline to post demonstrated a large effect in favour of CWI.

6.3.7.3. Peak torque 60°·s^{-1}

At baseline, peak torque values at 60°·s^{-1} were 237.3 ± 39.8 and 188.5 ± 29.8 Nm for PL and CWI respectively. Changes over time were trivial for PL at both time points, whilst CWI demonstrated a small increase, and then an unclear change at mid and post respectively. In terms of group comparison there was a large effect in favour of CWI at mid, but a trivial effect at post.

6.3.7.4. Peak torque 180°·s^{-1}

At baseline, peak torque values at 180°·s^{-1} were 167.1 ± 30.6 and 132.9 ± 12.9 Nm for PL and CWI respectively. There was a small increase for CWI from baseline to mid, but all other changes over time were unclear or trivial. As a result, group comparisons were unclear and trivial at mid and post respectively.

6.3.8. RSI

At baseline, RSI values were 1.66 ± 0.54 and 1.73 ± 0.38 cm·s^{-1} for PL and CWI respectively. Over time, changes in the PL group were unclear, and the CWI group demonstrated small increases at both time points. As a result, group comparisons were unclear at both time points.

6.3.9. Isometric peak force

At baseline, isometric peak force values were 1695.2 ± 310.3 and 1635.1 ± 598.3 Nm for PL and CWI respectively. Peak force values increased in both groups at all time points, however, group comparisons were unclear and trivial at mid and post respectively. Absolute change data are presented in Figure 6.2.

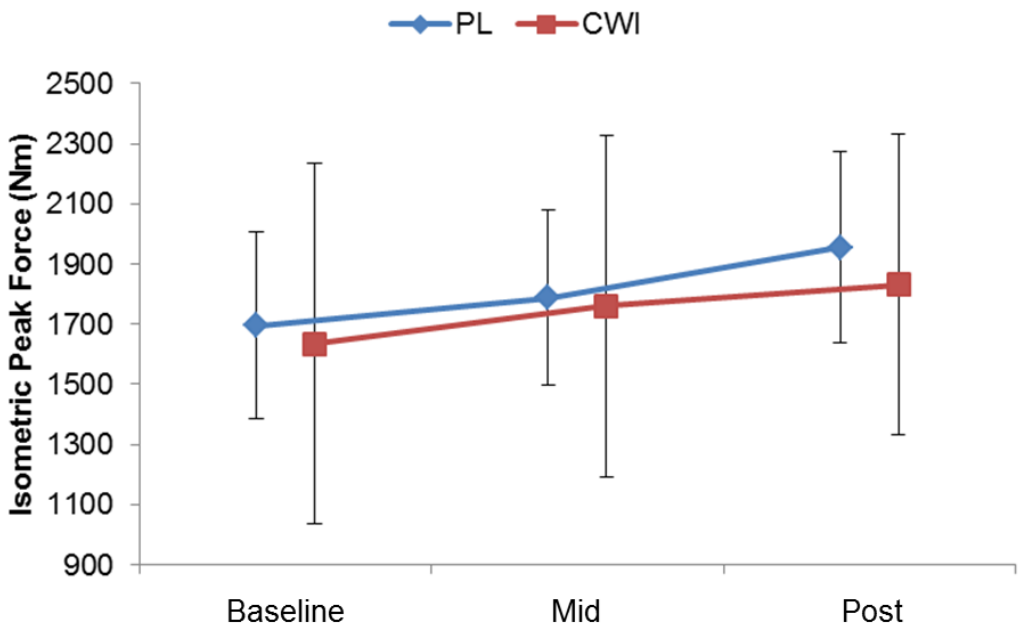

Figure 6.2. Changes in isometric squat peak force. Data are presented as mean ± SD.

6.3.10. RFD

6.3.10.1. RFD 50-100

At baseline, RFD 50-100 values were 5621.9 ± 2361.9 and 4241.9 ± 2119.7 $Nm \cdot s^{-1}$ for PL and CWI respectively. Changes for PL and CWI were unclear or small increases at mid and post respectively with unclear group comparisons.

6.3.10.2. RFD 100-200

At baseline, RFD 100-200 values were 5078.5 ± 1527.2 and 4172.9 ± 2119.2 $Nm \cdot s^{-1}$ for PL and CWI respectively. All changes over time and group comparisons were unclear for both groups.

Table 6.3. Change over time and group comparisons for ultrasound and functional markers

		Changes over Time Mean; ±CL ES Qualitative Outcome		Effects Mean[a]; ±CL[b] ES Qualitative Outcome
		Placebo	CWI	PL/CWI
Pennation Angle (%)	B – Mid	12.3; ±14.6 Trivial ↑**	10.9; ±11.2 Moderate ↑**	0.8; ±14.5 Unclear
	B – Post	21.5; ±8.4 Large ↑**	11.4; ±4.8 Moderate ↑***	-10.1; ±8.8 Moderate ↓**
MVIC 90º (%)	B – Mid	-0.1; ±8.9 ↔*	1.3; ±5.0 ↔**	1.4; ±9.5 Unclear
	B – Post	-4.4; ±8.0 Trivial ↓*	-1.1; ±7.6 ↔*	3.3; ±10.1 Unclear
MVIC 30º (%)	B – Mid	-3.4; ±10.0 Trivial ↓*	6.7; ±10.3 Unclear	10.1; ±13.2 Unclear
	B – Post	-7.9; ±10.1 Small ↓*	16.2; ±12.9 Small ↑**	24.1; ±15.1 Large ↑***
Peak Torque 60 deg·s (%)	B – Mid	-2.4; ±4.0 Trivial ↓**	7.5; ±6.5 Small ↑**	9.9; ±7.1 Large ↑**
	B – Post	1.6; ±5.5 ↔*	2.0; ±6.2 Unclear	0.4; ±7.6 ↔*
Peak Torque 180 deg·s (%)	B – Mid	2.8; ±4.4 Unclear	5.4; ±5.0 Small ↑*	2.6; ±6.1 Unclear
	B – Post	4.7; ±5.3 Trivial ↑*	1.9; ±4.8 ↔*	-2.8; ±6.5 ↔*
RSI (%)	B – Mid	13.4; ±20.6 Unclear	14.0; ±19.5 Small ↑*	0.6; ±26.0 Unclear
	B – Post	12.3; ±18.5 Unclear	13.5; ±15.7 Small ↑**	1.20; ±22.2 Unclear
Isometric Peak Force (%)	B – Mid	5.7; ±12.2 Unclear	8.4; ±11.5 Trivial ↑*	2.7; ±15.3 Unclear
	B – Post	15.8; ±14.4 Moderate ↑**	14.0; ±14.2 Small ↑**	-1.8; ±18.5 ↔*
RFD 50-100ms (%)	B – Mid	0.3; ±32.9 Unclear	34.0; ±43.1 Small ↑**	33.7; ±49.9 Unclear
	B – Post	17.9; ±32.2 Unclear	36.3; ±58.1 Small ↑**	18.4; ±62.1 Unclear
RFD 100-200ms (%)	B – Mid	0.4; ±28.6 Unclear	5.8; ±18.2 Unclear	5.4; ±31.6 Unclear
	B – Post	17.4; ±28.6 Unclear	14.9; ±39.2 Unclear	-2.5; ±44.7 Unclear

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease, and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

6.3.11. Back squat 4RM

At baseline, back squat 4RM values were 163.3 ± 42.3 and 139.2 ± 37.2 kg for PL and CWI respectively. Baseline values were compared to the load achieved in the final training session of the strength block. Change over time analyses revealed a small, and moderate, effect for PL and CWI respectively, which resulted in an unclear comparison between groups.

6.3.12. Unloaded and loaded CMJs

Percentage change values for jump height for all CMJ loads are displayed in table 6.4. For jump height at 0.1 kg, there was a trivial decrease for PL from baseline to mid, and a small decrease from baseline to post. For CWI there was a small decrease at both time points. For jump height at 20 kg, all changes were unclear with the exception of PL from baseline to post which showed a trivial change. For jump height at 50 kg PL demonstrated a trivial decrease and then an unclear change at mid and post respectively. Changes for CWI were trivial and unclear at mid and post respectively. Group comparisons were trivial or unclear at all time points with the exception of jump height at 50 kg from baseline to post, where there was a small effect in favour of PL.

Table 6.4. Change over time and group comparisons for back squat 4RM and CMJs

		Changes over Time Mean; ± CL ES Qualitative Outcome		**Effects** Mean[a]; ± CL[b] ES Qualitative Outcome
		Placebo	CWI	PL/CWI
Back Squat 4RM (%)	Session 1-8	18.8; ± 8.7 Small ↑***	20.3; ± 11.7 Moderate ↑***	1.5; ± 13.4 Unclear
Jump Height 0.1kg (%)	B – Mid	-5.5; ± 7.6 Trivial ↓*	-10.2; ± 10.8 Small ↓**	-4.7; ± 12.0 ↔*
	B – Post	-7.3; ± 9.1 Small ↓*	-10.3; ± 15.8 Small ↓*	-3.0; ± 16.8 ↔*
Jump Height 20kg (%)	B – Mid	6.8; ± 18.0 Unclear	6.3; ± 14.8 Unclear	-0.5; ± 18.0 ↔*
	B – Post	1.5; ± 8.2 ↔**	10.7; ± 29.9 Unclear	9.2; ± 30.8 Unclear
Jump Height 50kg (%)	B – Mid	-3.3; ± 8.7 Trivial ↓*	-1.1; ± 7.4 ↔**	2.2; ± 9.9 Unclear
	B – Post	7.6; ± 22.6 Unclear	2.0; ± 20.6 Unclear	-5.6; ± 26.5 Small ↓*

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group.[b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

6.3.13. Blood markers

6.3.13.1. PINP

At baseline PINP values were 130.2 ± 337 and 153.1 ± 49.8 ng/ml for PL and CWI respectively. From baseline to mid, both groups demonstrated a small decrease,

with a smaller decrease and therefore small effect in favour of PL. From baseline to post, the PL group demonstrated a trivial change whilst there was a small decrease in CWI, leading to a large effect in favour of PL.

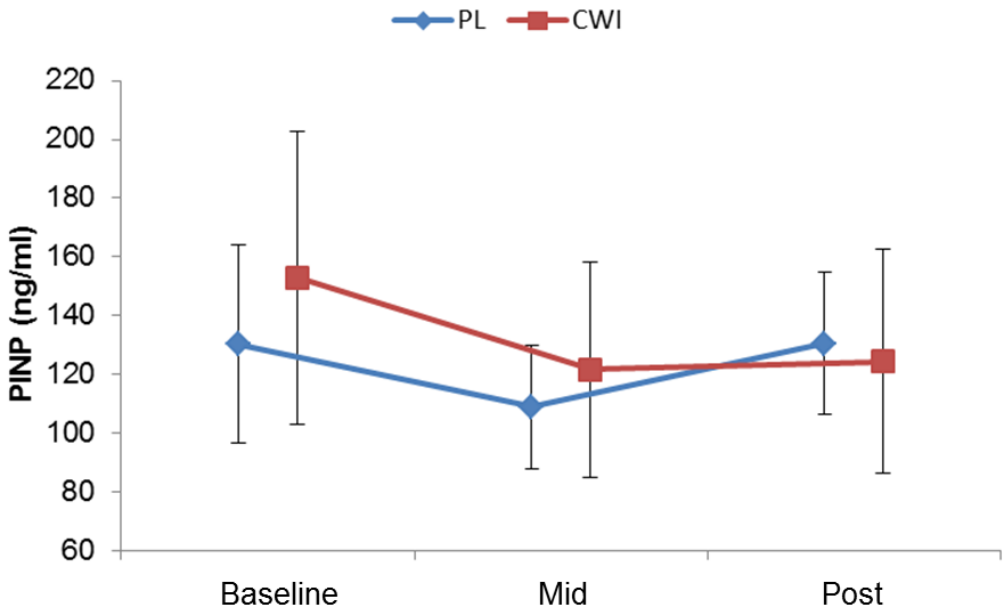

Figure 6.3. Absolute changes in PINP. Data are presented as mean ± SD

6.3.13.2. PIIINP

At baseline PIIINP values were 46.4 ± 6.1 and 49.4 ± 4.0 ng/ml for PL and CWI respectively. From baseline to mid, there were trivial changes in both groups, leading to an unclear group comparison. From baseline to post, there was an unclear change in the PL group, with a concurrent small decrease in the CWI group, resulting in a small effect in favour of PL.

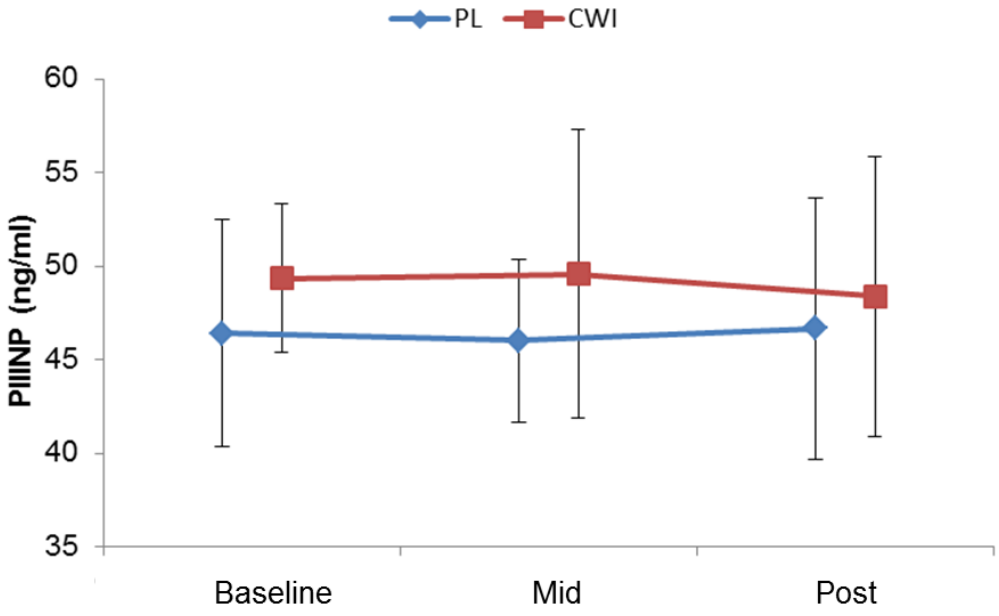

Figure 6.4. Absolute changes in PIIINP. Data are presented as mean ± SD.

Table 6.5 Change over time and group comparisons for blood markers and lean mass.

		Changes over Time Mean; x/÷ CL Qualitative Outcome		**Effects** Mean[a]; x/÷ CL[b] Qualitative Outcome
		Placebo	CWI	PL/CWI
PINP	B – Mid	0.846; x/÷ 1.093 Small ↓***	0.802; x/÷ 1.306 Small ↓**	0.95; x/÷ 1.312 Small ↓*
	B – Post	1.015; x/÷ 1.097 ↔**	0.819; x/÷ 1.226 Small ↓**	0.81; x/÷ 1.241 Large ↓**
PIIINP	B – Mid	0.995; x/÷ 1.081 Trivial ↓*	0.996; x/÷ 1.027 ↔**	1.00; x/÷ 1.083 Unclear
	B – Post	1.003; x/÷ 1.086 Unclear	0.974; x/÷ 1.073 Small ↓*	0.97; x/÷ 1.104 Small ↓*
Lean Mass (%)	B – Mid	0.3; ± 1.8 ↔***	0.4; ± 1.9 ↔***	0.1; ± 2.4 ↔***
	B – Post	0.0; ± 0.7 ↔**	-0.8; ± 1.7 ↔***	-0.8; ± 1.8 ↔***

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (*) with *possibly, **likely, ***very likely and **** most likely. For outcomes, ↑ is an increase, ↓ is a decrease , and ↔ is a trivial change. Where changes are trivial or unclear, no effect size is reported. [a]Mean represents the second named group minus the first named group. [b]90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference. For effect sizes, 0.0-0.19 is trivial, 0.20-0.59 is small, 0.60-1.19 is moderate, 1.20-1.99 is large, 2.0-3.99 is very large and 4.0+ is extremely large.

6.4. Discussion

The training programme implemented as part of this study resulted in improvements in back squat 4RM for both groups with no difference between groups. A hypertrophic response was apparent in both groups, evidenced by increases in fibre pennation angle, although there was no overall change in total lean mass in either group. There were also clear increases in RSI, isometric peak force, peak torque at 180°·s^{-1}, and RFD. There was no positive effect of training on peak torque at 60°·s^{-1}, isometric force production or CMJ.

The overall findings from this study suggest that although some functional outcomes improved to a greater extent following CWI, this did not always result in clear effects in terms of group comparisons. Conversely, CWI appeared to attenuate hypertrophy measured via changes in pennation angle, and the results suggest that habitual CWI exposure may inhibit some of the physiological processes associated with the rate of collagen and bone turnover. In terms of perceptual responses, group comparisons were unclear, or favoured the placebo condition.

The angle of fibre pennation in the VL increased in both groups at both time points. Whilst group comparisons were unclear from baseline to mid, there was a moderate effect in favour of placebo from baseline to post. These results indicate that repeated CWI may interfere with the process of muscle protein synthesis and negatively impact upon resultant skeletal muscle accretion. The findings from the present study are in line with those from Roberts, Raastad, et al. (2015) who demonstrated that type II muscle fibre cross-sectional area and the number of myonuclei per fibre increased in the active recovery group, (all $P<0.05$), but not the CWI group following a 12 week strength training intervention. Muscle biopsy data from a follow up study revealed that CWI attenuated acute changes in satellite cell numbers and activity of kinases (NCAM^{+} and Pax7^{+}) that regulate muscle

hypertrophy, which may have resulted in the diminished long-term training gains in muscle hypertrophy for the CWI group (Figueiredo et al., 2016).

Changes in MVIC at 90° were trivial in both groups, leading to unclear group comparisons. However, for MVIC at 30°, there was a small decrease, and a small increase for placebo and CWI respectively, from baseline to post, leading to a large effect in favour of CWI for this parameter. It is possible that these findings are related to the observed changes in muscle architecture. The angle at which fibres are oriented relative to the axis of force generation will determine the proportion of fibre tensile force that is transmitted to the tendon (Lieber & Friden, 2000; Maffiuletti et al., 2016). Given that participants in the placebo group demonstrated larger increases in pennation angle, a small proportional reduction in the amount of force transmitted to the tendon may offer an explanation for the observed results. This hypothesis could also explain the results from isokinetic testing; group comparisons for peak torque were trivial or unclear with the exception of peak torque at $60°{\cdot}s^{-1}$ from baseline to mid, where there was a trivial decrease for PL and a small increase for CWI, leading to a large effect in favour of CWI.

Further, these findings suggest that measures of hypertrophy cannot be used to infer concurrent improvements in function (Siff, 2003), and that neural adaptations are likely to account for a significant proportion of strength development (Carroll et al., 2001). Alternatively, it may be that the use of isometric and isokinetic dynamometry demonstrates limited transferability to functional performance (Byrne et al., 2004; Cockburn et al., 2013) and that the more sport specific measures recorded in this study may be more appropriate markers.

In terms of some of the more dynamic and sport specific markers, RSI improved in both groups throughout the training intervention with a greater increase in CWI, however group comparisons revealed unclear effects. Unlike some of the peak

torque markers which are directly related to absolute muscle strength, improvements in RSI are more likely to be attributed to neural adaptations. Similarly, data derived from the isometric squats (peak force and RFD) demonstrated unclear group comparisons, but CWI elicited greater improvements than PL in peak force from baseline to mid, and in all RFD parameters. Again, the results demonstrate that training resulted in improved RFD scores irrespective of the recovery modality. For the unloaded and loaded CMJs, changes over time for jump height were unclear, trivial or showed small decreases for both groups, across all loads, at both time points. For jump height at 50 kg there was a small effect in favour of PL, however all other group comparisons were trivial or unclear. There were large confidence limits for all the jump variables, which likely contributed to the unclear and trivial outcomes. It may have been more appropriate to individualise loads for each participant based on a percentage of body mass, lean mass or a predicted 1RM, in order to account for strength variations within groups.

The finding that CWI did not attenuate the magnitude of soreness experienced by participants from week 1 to 4 was unexpected. Further, participants in the placebo group appeared to experience diminished soreness responses from week 1 to week 4 compared to CWI. There is a wealth of literature relating to the phenomenon of the repeated bout effect, whereby one bout of eccentric exercise protects against muscle damage from a subsequent bout (Barnett, 2006; McHugh, 2003; McHugh et al., 1999; McKune et al., 2012; Proske & Allen, 2005; Stupka, Tarnopolsky, Yardley, & Phillips, 2001). It is possible that CWI exposure may have altered blood flow and/or oxygen and nutrient delivery to skeletal muscle, meaning that participants in the CWI group did not benefit from the full protective consequence of the repeated bout effect in subsequent sessions. Findings from the perceptions of recovery questionnaire echo the soreness data; group comparisons were trivial or unclear, but from pre to 48 h post from week 1 to 4, the CWI group showed a smaller

improvement in recovery scores. Again, this is not in agreement with the majority of acute studies that show improved perceptual recovery following cryotherapy compared to control interventions (Fonda & Sarabon, 2013; Garcia et al., 2016; Hausswirth et al., 2011). Similarly, DALDA and sleep efficiency data revealed unclear or trivial group comparisons, with predominantly unclear or trivial changes over time. Sleep has numerous important physiological functions that are particularly pertinent to athletes, such as allowing recovery from previous wakefulness, and preparing for functioning in the subsequent wake period (Halson, 2014b). These data suggest that whilst cryotherapy does not negatively impact upon stress reaction symptoms or sleep efficiency, it does not offer any additional benefits.

PINP and PIIINP are markers of bone turnover and collagen formation respectively and can be measured from blood, without a need for biopsy techniques. Although changes over time in both groups revealed trivial changes or small decreases for PINP, group comparisons revealed small and large effects in favour of placebo at mid and post respectively, indicating that cryotherapy may interfere with, or slow down, some of the processes relating to bone turnover to a greater extent compared to a placebo intervention. Similarly, there was a small effect in favour of placebo for PIIINP from baseline to post. It is worth noting that although both PINP and PIIINP are relatively sensitive markers, intra-individual variations can be relatively large; 13-15% and 12-19% respectively (Erotokritou-Mulligan et al., 2010; Nguyen et al., 2008). Whilst the changes in these markers remained relatively small (and well below clinical significance), it is possible that habitual cryotherapy use over a longer period of time may lead to more pronounced reductions in these markers, negatively impacting upon the physiological processes of repair and adaptation. Previous studies investigating the impact of training on these markers has shown that in acute investigations, markers of bone formation tend to decrease (Banfi, Lombardi,

Colombini, & Lippi, 2010). However, this attenuation appears to be transient as recent longitudinal studies tend to show no significant change following endurance (Cornelissen et al., 2010; Zanker & Swaine, 2000) or resistance (Bloomquist et al., 2013; Sartorio et al., 2001) training. PINP and PIIINP are not routinely reported in the cryotherapy literature, so the inclusion of these markers in the current study represents a novel addition. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) stimulate osteoblast proliferation and both play a significant role in the regulation of growth and bone metabolism (Xian et al., 2012). Previous research in rats has demonstrated that cryotherapy application post injury attenuated the expression of type I and III collagens, as well as growth factors responsible for their stimulation including IGF-1, most markedly at 3 days post lesion (Vieira Ramos et al., 2016). Therefore, the larger reductions in PINP and PIIINP observed in the cryotherapy group in the current study could be attributed to this mechanism.

Potential limitations of the current study should be addressed. Low participant numbers in each group reflect the substantial time commitment required for the study; volunteers who completed the intervention were involved for approximately 3 months in total. Secondly, as already mentioned, the loads used for the CMJs were not individualised, and this may have diluted any potential treatment and/or training effects. Participants were matched into groups based on lean mass and 4RM, and as a result there were some differences in absolute strength between groups. However, all functional outcomes were reported as percentage change to minimise the potential confounding effect of absolute raw values. Although muscle fibre pennation angle was included as a measure of hypertrophy, the training programme implemented was targeted towards strength and power. As such, a hypertrophy programme may have been more efficacious in affecting morphological changes. Lastly, the addition of more ecologically valid sport specific outcome measures may have increased the applicability of the findings to practice, however, as with the

majority of sport science research, there is always a compromise between internal and external validity (Atkinson & Nevill, 2001).

The results from the study indicate that hypertrophy was evident in both groups, but it is likely that the relatively short intervention resulted in largely neural adaptations to training. The findings from this study suggest that repeated CWI does not accentuate functional adaptations to resistance training, despite a possible benefit for neural adaptation, and may even result in diminished hypertrophic and perceptual responses. The shorter training programme (2 x 4 weeks) utilised in the present study is a novel addition to the literature. Similarly, the finding that CWI may enhance neural adaptation in the early stages of a strength and power training programme is an important and novel finding. Where a longer training programme (12 weeks) has been utilised (Roberts, Raastad, et al., 2015), CWI attenuated long term gains in muscle mass and strength. This may indicate that any early neural benefit of CWI is negated when hypertrophy begins to play a role, and could be linked to the negative impact of CWI on markers of bone and collagen turnover. It is therefore likely that there is a cumulative impact of cryotherapy on adaptation, and that greater decrements may be seen as a result of sustained exposures.

6.5. Conclusion

The overall aim of the thesis was to add to the current body of knowledge relating to the effects of acute and habitual cryotherapy exposure on recovery and adaptation following strenuous exercise, using placebo-controlled study designs. In terms of the specific thesis aims, this study addressed aim 4 (to investigate whether repeated cryotherapy exposure during an ecologically valid strength and power training programme influences training adaptations compared to a placebo intervention). The findings from this study offer novel evidence that CWI may negatively influence bone and collagen turnover in the early phase of a strength and

power training programme. Additionally, skeletal muscle hypertrophy may be attenuated potentially leading to functional impairment over time. Strength training is an important adjuvant to specific skills training in a variety of sports; therefore, the widespread use of cryotherapy by individuals hoping to improve athletic performance should be reconsidered.

Chapter 7

General Discussion

7.1. Contribution of findings to the literature

The overall purpose of this thesis was to investigate the impact of cryotherapy (CWI or WBC) or a placebo intervention on recovery following strenuous exercise. The studies investigated acute recovery following different exercise stresses, as well as the impact of habitual CWI exposure on adaptations to strength and power training. The findings suggested that in terms of acute recovery following resistance training, although WBC may offer some small functional and perceptual benefits over CWI at 24 h, the majority of outcomes were trivial or unclear and WBC exacerbated the response of several blood parameters compared to CWI. However, after endurance exercise WBC is less favourable than CWI for recovery of functional, perceptual and blood parameters. Furthermore, the findings from both acute studies suggest that neither cryotherapy treatment is more effective than a placebo condition, indicating that treatment belief and the placebo effect may be largely responsible for much of the positive evidence reported in the literature (Ascensão et al., 2011; Lombardi et al., 2017; Poppendieck et al., 2013; Rose et al., 2017). Given the enduring popularity of CWI and its ease of application (Ihsan et al., 2016), the final study of this thesis investigated the influence of repeated immersion on adaptations to an ecologically valid resistance training protocol. The findings revealed that habitual CWI exposure in conjunction with a structured (2 x 4 week) strength and power training programme may result in diminished hypertrophy and perceptual responses as well as attenuated collagen and bone turnover compared to a placebo intervention. Interestingly, despite this, CWI appeared to result in larger improvements in a number of functional markers (possibly due to improved neural adaptations), although this did not translate to clear effects when compared to PL, and the potential mechanism(s) remain unclear.

There are a number of proposed theories to support the use of cryotherapy as an acute recovery intervention following strenuous exercise. Aside from the potential

analgesic effects arising from cold exposure (Bleakley et al., 2012; Ihsan, Watson, & Abbiss, 2016; Lombardi, Ziemann, & Banfi, 2017; Nadler, Weingand, & Kruse, 2004), cryotherapy may also blunt the acute inflammatory response initiated by strenuous exercise by modulating blood flow (Mawhinney et al., 2013), which could theoretically limit undesirable alterations in functional, perceptual and blood borne markers. However, the findings from the first two studies in this thesis directly contradict this theory, with evidence to suggest that cryotherapy exposure actually exacerbated the inflammatory response in some cases.

As already discussed, there is an increasing amount of evidence to suggest that many of the beneficial effects associated with cryotherapy are likely to be due to the placebo effect (Broatch et al., 2014; McClung & Collins, 2007; McGorm et al., 2015; Minett & Costello, 2015; Poppendieck et al., 2013). Cryotherapy application poses a unique challenge in terms of blinding participants to their interventions. As a result, the majority of existing cryotherapy literature (Crystal, Townson, Cook, & LaRoche, 2013; Ferreira-Junior et al., 2015; Fonda & Sarabon, 2013; Higgins, Cameron, & Climstein, 2012; Krüger, de Mareés, Dittmar, Sperlich, & Mester, 2015; Pointon, Duffield, Cannon, & Marino, 2012; Roberts, Nosaka, Coombes, & Peake, 2015) fails to employ an effective placebo condition. The studies in this thesis aimed to address this problematic issue by utilising appropriate sham treatments. Sham treatments were based on interventions that have proven effective in past research relative to the specific exercise stress employed in each investigation. It was hoped therefore, that expectance effects would not be disproportionate between groups, although this was not explicitly measured. The findings from the acute studies support the argument that the success of cryotherapy treatment may be, at least partly, related to a placebo effect. Although CWI and WBC attenuated increases in muscle soreness and stress reaction symptoms, a novel finding was that there were was little benefit in terms of functional recovery compared to the placebo interventions.

Furthermore, the rise in popularity of WBC has occurred in the absence of a wealth of literature supporting its use (Banfi, Lombardi, Colombini, & Melegati, 2010; Holmes & Willoughby, 2016). Literature relating to use of WBC in an applied performance setting remains scarce and there is also a dearth of literature comparing the different modalities. Indeed, at the commencement of this project, there was no literature directly comparing functional recovery outcomes following WBC and CWI exposure. By directly comparing the two different cryotherapy modalities (CWI and WBC), the findings from the studies within this thesis go some way to addressing a gap in the current body of literature. Although there is an abundance of CWI literature, and a growing body of WBC studies, methodological differences mean it is difficult to compare findings and make objective recommendations using available data. The applied focus of this thesis dictated that ecologically valid exercise protocols were implemented in all studies, allowing for real world application of the findings. Furthermore, there was a need to consider recovery in a holistic sense, rather than focusing on single facets (function *or* perceptions *or* blood borne markers) as has been done previously (Banfi, Melegati, Barassi, & d'Eril, 2009; Machado et al., 2016; Peake et al., 2017; Pournot et al., 2011). The findings from the acute studies demonstrated that the effectiveness of CWI and WBC was influenced by exercise mode, reiterating the need for specificity when selecting recovery interventions post exercise.

7.2. Acute perceptual outcomes

A number of previous studies have reported beneficial effects of cryotherapy treatment on acute perceptual recovery following strenuous exercise (Bleakley et al., 2012; Cheung et al., 2003; Rose et al., 2017). Many of the studies reporting positive findings have utilised passive recovery or control groups; the studies in the current thesis employed placebo-controlled designs in an attempt to address this constraint and improve scientific rigour. In terms of perceptual recovery, WBC was more

beneficial than CWI in relation to stress reaction symptoms in both of the acute studies, but was only superior for the attenuation of soreness 24 h following the resistance exercise bout. Given that CWI is a far more efficient method of cooling than WBC (Holmes & Willoughby, 2016; Ihsan et al., 2016; Tipton et al., 2017), it is possible that participants felt more discomfort during CWI than WBC, leading to the favourable WBC outcomes for stress reaction symptoms across both studies. Secondly, although not specifically measured within the studies, it is possible that the prolonged endurance exercise bout resulted in a greater thermal strain and rise in core temperature than the acute resistance training session; whilst anecdotal evidence would suggest that participants felt refreshed or invigorated after WBC exposure, a 1 x 3- and 1 x 4 min exposure at -85°C following prolonged running may have been insufficient to affect a decrease in tissue temperature that could mitigate delayed onset muscle soreness. This may explain the differing soreness responses between the endurance and resistance exercise bouts. As has been previously mentioned, the treatment temperature used for the acute studies within this thesis was warmer than that normally reported in the literature (-85° compared to -110°C), and this should be considered when comparing findings from the present investigation to previous literature.

Furthermore, and in contrast to the majority of published literature, cryotherapy (either CWI or WBC) was not always more effective than the placebo intervention for attenuating soreness or stress reaction symptoms. These findings are however supported by Broatch, Petersen and Bishop (2014) who stated that a placebo intervention administered following high-intensity interval training was as effective as CWI. The findings from this thesis further reinforce the need for future cryotherapy studies to implement effective blinding procedures, or account for expectance effects.

7.3. Acute functional outcomes

The effectiveness of the cryotherapy interventions for the recovery of functional outcomes from the acute studies in this thesis appeared to be differently affected by the exercise stress employed. For the endurance study, WBC was less effective than both CWI and PL across all measured functional outcomes, whereas for the same outcomes following resistance exercise, although CWI was less effective than WBC for MVIC at 90°, both CWI and WBC were less effective than PL for peak torque and RSI measures. These findings further support the suggestion that cryotherapy treatment is not, and cannot be, a one-size fits all solution (Minett & Costello, 2015; Stephens et al., 2016).

Although not directly measured as part of this thesis, CWI and WBC are not equal in terms of their capacity to bring about temperature reductions; a recent study from Mawhinney and colleagues (2017) revealed that greater reductions in blood flow and tissue temperature are elicited following CWI compared to WBC. WBC appears to be less effective than CWI where there is likely to be a large proportion of metabolic damage and resultant heat generation (White & Wells, 2013), and this may be due to the diminished cooling capacity and blood flow attenuation compared to CWI (Mawhinney et al., 2017). As previously stated, the CWI immersion temperature changed from 8°C in study 1, to 10°C in study 2 in response to published recommendations. However, research from Vaile et al. (2011) demonstrates that immersion at 15°C is sufficient to bring about a significant decrease in limb blood flow following exercise, suggesting that an increase from 8 to 10°C in the current investigation is likely to have little impact in terms of modulating temperature and blood flow.

The finding from this thesis that cryotherapy (either CWI or WBC) offers little benefit for functional recovery, challenges a proportion of the previous literature (Ascensão

et al., 2011; Ferreira-Junior et al., 2015; Ingram et al., 2009; Krüger et al., 2015; Montgomery et al., 2008; Pournot et al., 2011; Santos et al., 2012; White et al., 2014). Recent reviews from Higgins, Greene and Baker (2017) and Rose et al. (2017) reported that CWI and WBC respectively were beneficial for functional recovery following strenuous exercise. However, all of the studies included in the analyses used either no specific control group, or a passive recovery intervention by way of a control, and results should therefore be interpreted with caution. Rose et al. (2017) highlighted this as a limitation and stated that it was therefore impossible to determine the potential contribution of a placebo effect. The contradictory findings from the current thesis could be partly attributed to improved methodological rigour in the present studies; by utilising a relatively novel placebo controlled study design, it was possible to remove, or at least limit the influence of any potential placebo effects on the measured outcome variables. The findings lend further weight to the argument that previous beneficial findings may be confounded by the placebo effect in non-blinded studies (Broatch et al., 2014).

Additionally, it is possible that methodological differences between studies could contribute to the disparate findings. As has already been highlighted, the response to any cryotherapy intervention is influenced by the exercise stress itself, and with innumerable exercise stresses utilised in the literature, it is difficult to generalise findings. Furthermore, there is huge variation in cryotherapy protocols, with different temperatures, durations, number of exposures, and methods of application reported in previous investigations (Bleakley et al., 2012). This is likely to result in different magnitudes of cooling which would in turn influence the impact of each intervention. Lastly, the functional markers used to determine the recovery of performance can be as varied as the exercise stresses. As discussed in the previous chapter, the selection of functional outcome measures is also likely to influence the findings. In

order to be practically relevant, functional markers should be appropriate to the exercise stress, which means that findings may not be comparable across studies.

7.4. Acute inflammation and muscle damage responses

A further specific area of study is the influence of cryotherapy on markers of inflammation or fluctuations in the efflux of intracellular proteins following muscle damaging exercise. The findings from the studies within this thesis demonstrated that cryotherapy did not attenuate levels of circulating CK or IL-6 at any point post exercise compared to the placebo conditions. These findings are in accordance with a systematic review and meta-analysis from Hohenauer et al. (2015) which revealed no significant effect of cooling on levels of circulating CK. Similarly, the analyses revealed no significant impact of cooling on IL-6.

Given that structural damage and acute inflammation are initiated during strenuous exercise, rather than at the cessation of activity, any intervention applied post exercise, may have little influence on these physiological alterations. Where beneficial effects have been reported in the hours immediately after exercise (Santos et al., 2012), it is possible that a transient alteration in blood flow (Mawhinney et al., 2013, 2017) may slow the movement of these markers from the muscle to the peripheral circulation. Furthermore, it is possible that any 'benefit' seen immediately post cryotherapy treatment is negated when blood flow returns to normal, and that despite decreased peak values, the return to baseline levels may simply be delayed. In other words, the area under the curve is comparable, but the curve itself is flattened as a result of cryotherapy treatment.

Alternatively, as previously discussed, acute severe cold exposure can also be interpreted by the body as a source of stress, resulting in an exacerbation of the inflammatory response (Machado et al., 2016). This was evidenced in both the acute studies of this thesis: in the endurance study, CRP was elevated at 24 h in the

WBC group compared to placebo, IL-6 was elevated immediately-, and 24 h post for CWI and WBC respectively compared to placebo, and both cryotherapy conditions exacerbated TNF-α immediately post intervention. Similarly, in the acute resistance study TNF-α was increased by both cryotherapy conditions compared to placebo at the majority of time points. This could therefore explain the lack of positive findings associated with cryotherapy in relation to an attenuated inflammatory response, despite literature evidencing the modulation of blood flow following exposure (Gregson et al., 2011; Houben et al., 1982; Khoshnevis et al., 2015; Mawhinney et al., 2013, 2017).

However, in terms of the attenuation of CRP, the review from Hohenauer et al. (2015) revealed that cryotherapy significantly attenuated increases in CRP 48 h post exercise, although the authors stated only 4 studies with a total of 65 volunteers accounted for the significant finding. The authors further suggested that future studies should employ blinding strategies to limit bias. The current thesis attempted to address this issue and the acute studies demonstrated that CWI resulted in an attenuated CRP response (24-48 h) irrespective of exercise stress. However, responses differed between cryotherapy modalities; in the endurance study WBC resulted in a greater CRP response compared to CWI and placebo at 24 h, and in the acute resistance study, WBC exacerbated circulating CRP at all time points compared to CWI, and at 48 and 72 h compared to the placebo.

The differences may be explained in part by the CRP response observed after different exercise stresses, but the findings also suggest that, as previously mentioned, cryotherapy itself may represent a cold stress that modulates the inflammatory response (Machado et al., 2016). Research has shown that WBC does not have the same capacity as CWI to affect a significant reduction in blood flow (Mawhinney et al., 2017), therefore, WBC may exacerbate the inflammatory response, without the concurrent reduction in blood flow evidenced following CWI,

leading to inflated levels of inflammatory markers such as CRP. These findings further support the suggestion that although cryotherapy applied post exercise may be ineffective at reducing the IL-6 response, as CRP levels continue to rise post exercise, often peaking at 24h, CWI application post exercise may influence or attenuate some of the physiological mechanisms associated with increased levels of CRP.

The overall findings from the acute studies within the current thesis challenge the notion that WBC is a superior recovery intervention in comparison to CWI (Bleakley et al., 2014). Whilst WBC offered some perceptual benefit compared to CWI and PL at certain time points, this did not often translate to favourable performance outcomes. This could be attributed to the WBC protocol utilised in the acute studies; it is possible that a treatment temperature of -85°C combined with the poor thermal conductivity of air results in suboptimal cooling of subcutaneous tissues.

7.5. Adaptation

Although the findings from the acute studies offer little evidence to support the use of cryotherapy as a recovery strategy, CWI in particular remains a popular intervention amongst athletes (Ihsan et al., 2016). Where exposure guidelines are adhered to (in terms of appropriate health screening and temperature monitoring), the available literature suggests that there are unlikely to be any lasting deleterious health implications of acute CWI exposure (Lombardi et al., 2017). However, for athletes regularly using cryotherapy as an integral part of their training and recovery cycle, the influence of repeated exposures on long term adaptations to training remains a key consideration.

The final study of this thesis examined the impact of habitual CWI exposure on adaptations to strength and power training. Although it was not possible to take biopsy samples as part of this thesis, the smaller increases in pennation angle

evidenced in the CWI group during the training study indicate that cryotherapy exposure can lead to diminished or maladaptive strength training responses. This finding is supported by recent work from Roberts et al. (2015) who demonstrated that repeated CWI exposure resulted in diminished satellite cell activity, leading to potentially reduced longer term strength and power gains. However, the diminished hypertrophic response was not reflected in the functional responses, where despite largely trivial or unclear group comparisons, CWI demonstrated greater improvements in a number of markers than PL. This in turn may indicate that CWI can in some way enhance neural adaptations to strength and power training, although the potential underpinning mechanisms require further investigation. These findings add to the current body of literature, by demonstrating that in as little as 8 weeks, habitual CWI exposure can modulate physiological adaptations to training. However, the potential negative impact of CWI may not become functionally apparent until the diminished hypertrophic responses outweigh short term neural benefits.

An important finding from the present investigation was that soreness responses were negatively impacted by repeated CWI. As already alluded to, many previous acute recovery investigations, including those in this thesis, have reported beneficial effects of cryotherapy on perceptual recovery post exercise, often in the absence of any other functional benefit. As such, athletes and coaches may choose to continue using cryotherapy to alleviate soreness, however, based on the results from the final study of this thesis, it may be appropriate for athletes to reconsider whether the potential short term gains justify the potential for longer term maladaptation.

In addition to the previously reported changes in satellite cell activity (Broatch et al., 2018; Roberts, Raastad, et al., 2015), other physiological mechanisms could also contribute to the findings in the current thesis. Research has shown that both WBC (Selfe et al., 2014) and CWI (Mawhinney et al., 2013; Vaile et al., 2011) lead to

alterations in blood flow, and that the effect is more profound following CWI (Mawhinney et al., 2017). As already suggested, a decrease in muscle blood flow may lead to an impairment of oxygen and nutrient transportation and utilisation which could ultimately be detrimental to recovery (Ihsan et al., 2016). Whilst satellite cell activity has been examined following cryotherapy exposure, the inclusion of specific bone and collagen markers in the training study gives novel insight into the underpinning physiological mechanisms at play and adds new knowledge to the current body of literature. Measures of PINP and PIIINP were recorded throughout the training study to assess any potential impact of CWI on bone and collagen turnover. The findings suggest that although turnover decreased slightly in both groups, a greater decrement was observed in the CWI group, which could indicate a maladaptive response to the training stimulus. Although it was not reflected in the functional outcomes, (where CWI actually enhanced some markers compared to PL) it may be that over a longer period of time, CWI may negatively impact upon structural and physiological adaptations to training, resulting in diminished functional gains.

The findings of this thesis could have practical implications for any athletes or practitioners implementing cryotherapy as a recovery modality following strenuous exercise. Generally, the findings do not support the use of cryotherapy interventions to accelerate recovery following exercise, either as an acute or chronic treatment.

7.6. Strengths, limitations and future directions

This thesis was applied in its direction and aimed to take a holistic approach to the multifaceted issue of recovery and performance. One of the key findings from this thesis is that neither CWI nor WBC offers enhanced recovery benefits compared to a placebo intervention. As already discussed, very little research conducted on cryotherapy application has effectively blinded participants to their treatment

intervention. By utilising a placebo-controlled study design in all of the investigations contained in this thesis, many of the study outcomes were less favourable in relation to cryotherapy than some previous investigations (Ascensão et al., 2011; Ferreira-Junior et al., 2015; Higgins et al., 2012; Krüger et al., 2015; Pournot et al., 2011). The use of recovery strategies is widespread amongst athletes of all levels (Barnett, 2006), therefore it may be useful if future research focused on the use of one intervention compared to another, rather than comparing one intervention to the absence of any intervention, as this is unlikely to be representative of current practices amongst athletes (Vaile et al., 2010).

A further strength of the thesis comes from the inclusion of markers not commonly utilised in sport science research. By including PINP and PIIINP as well as analysis of ultrasound images, it is hoped that the investigations herein may offer additional insight relating to physiological mechanisms that may be influenced by repeated cryotherapy exposure.

Whilst the findings from this thesis have furthered the understanding of cryotherapy treatment in relation to recovery, there are a number of limitations that should be addressed. Firstly, whilst the use of a placebo-controlled design is relatively novel and may have improved methodological rigour compared to previous investigations, the study design could have been further improved with the addition of a true control group. Whilst implementation of a placebo group may negate some of the issues surrounding placebo effects, including an additional control group would have strengthened the findings. Furthermore, it would have been useful to have a ‘measure’ of athletes’ perceptions of the efficacy of the proposed intervention (Venter, 2012), in order to better understand the contribution of expectance effects.

A further limitation of the current investigation, although not unusual in sport science research, is the relatively low power due to small sample sizes and multiple

comparisons. Although there remains some debate about the appropriateness of MBI statistical approaches, by utilising them here it was hoped that it would still be possible to determine practically meaningful results (Batterham & Hopkins, 2006). It should be noted that if all the data were reanalysed using NHST's it is likely that many of the trivial and small findings would be 'insignificant'.

It has already been acknowledged that the WBC protocol utilised in the first two studies of this thesis represents a warmer treatment temperature than that which is commonly used in practice. This was largely constrained due to the operating temperature of the electronically controlled chamber employed in study 1, therefore there is room to examine whether comparable results would be elicited if participants were exposed to the colder temperatures (-110 to -195°C (Abaïdia et al., 2016)) normally used in practice.

Similarly, muscle function variables in this thesis were selected based upon previous investigations, and their transferability to sporting movements, but concessions were made to try and maintain the integrity of data collected. As an example, in the case of study 1, the inclusion of a time trial variable may have provided further insight into the impact of the selected interventions on recovery, although completion of a maximal/exhaustive protocol would likely have impacted on subsequent data collection sessions. Given previous research indicating that CWI may impact upon muscle power and muscle strength differently (Leeder et al., 2012), the selection of muscle function outcomes will likely influence study findings.

Finally, throughout the thesis, the role of inflammation in the process of both muscle breakdown and regeneration has been discussed (Toumi & Best, 2003). Although inflammatory markers were measured in both of the acute studies, there was scope to include more specific markers that may have offered greater discernment in relation to underlying physiological mechanisms Similarly, the inclusion of a

measure of ROS could have strengthened the findings. Measurement of these additional markers of interest was not possible due to funding limitations. The inclusion of such markers in future investigations would add valuable knowledge to the current body of literature.

The findings from this thesis have added to the current body of literature but there are also a number of potential avenues for future enquiry: further applied research, further examination of placebo and expectance effects, and investigating potential training status and sex differences. In terms of applied research, there is still scope to develop specific CWI and WBC best practice guidelines. Despite a growing body of literature, optimal cryotherapy protocols have yet to be established (White & Wells, 2013). Whilst there are broad recommendations for CWI application (5-20°C for 5-30 min (Vieira et al., 2016)), guidelines are not specific to exercise mode, underpinning mechanisms or participant characteristics (Minett & Costello, 2015). The findings from this thesis demonstrate that exercise mode influences the response to cryotherapy exposure, and a recent investigation from Zandvoort, de Zwart, van Keeken, Viroux and Tiemessen (2018) demonstrated that customised CWI protocols outperformed standardised CWI protocols for the recovery of cardiovascular and neuromuscular performance following 60 squat jumps and an exhaustive 150 min cycling time trial.

An additional confounding variable is that of expectance effects; that is, the degree to which an individual believes that a treatment or intervention will work (McClung & Collins, 2007). One possible way to take into account the impact of expectance effects would be to survey participants in terms of their perceptions regarding a variety of recovery interventions. Whilst such surveys have been conducted amongst many different athlete populations (McClung & Collins, 2007; Venter, 2012), at the time of writing, it does not appear that this kind of information is routinely included as part of applied recovery research using traditional ‘treatment

vs control' study designs. If treatment belief was markedly greater for a specific strategy or intervention, it may be possible to take this into account when reporting results. Similarly, where treatment belief is high, even in the absence of any proven ergogenic effect, an intervention could still be an effective performance or recovery aid (McClung & Collins, 2007).

All participants included in this thesis were moderately trained males regularly completing endurance or resistance based training and therefore study findings can be generalised to these groups. However, it is not possible to extrapolate findings to highly trained or elite individuals, or females. Although some cryotherapy research has been carried out using highly trained individuals (Al Haddad et al., 2012; Ascensão et al., 2011; Bahnert et al., 2013; Russell et al., 2017; Selfe et al., 2014), methodological issues relating to standardisation of exercise stress, blinding, and sample size means the results should be interpreted with caution. Furthermore, there is evidence to suggest that genetic variations may influence an individuals' response to muscle damaging exercise (Baumert, Lake, Stewart, Drust, & Erskine, 2016), making it even more difficult to generalise findings. Given the current popularity of cryotherapy, and particularly WBC, amongst top level clubs and teams, further research with elite populations is warranted.

Further, one area that is lacking in sport science research, is investigations utilising female athletes (Costello, Bieuzen, & Bleakley, 2014). This may be due in part to a smaller pool of female athletes compared to males (although participation at a recreational level is approximately equal) (Costello et al., 2014); however physiological differences between males and females may represent further confounding factors in muscle damage and recovery research. A number of studies suggest that there may be gender-based differences in skeletal muscle responses to EIMD. In both human and animal studies, females have been shown to have lower CK following EIMD and it has therefore been hypothesised that females may

sustain less damage (Kendall & Eston, 2002). Despite evidence suggesting that oestrogen, a powerful antioxidant, can facilitate membrane stability and therefore limit CK leakage (Tiidus, 2000), some histological studies have shown no differences between male and female muscle following damage. Furthermore, and of particular pertinence to cryotherapy research, females tend to have a higher body fat percentage than men for a given body mass (Power & Schulkin, 2008), as well as different patterns of fat distribution. Given that subcutaneous fat insulates the body and inhibits heat loss, any potential therapeutic impact of cryotherapy is likely to be influenced by levels of adipose tissue. Therefore, investigating gender differences (in terms of whole body or segment specific fat mass percentages) and individualising treatment (in terms of temperature and duration) to optimise perceptual and functional recovery is a logical next step.

Lastly, the final study of this thesis focused on CWI as it remains a more common and widely used modality of cryotherapy than WBC. However, further research is warranted to examine the influence of repeated WBC exposure on recovery, performance and the processes of adaptation after exercise (Rose et al., 2017).

7.7. Conclusion

In conclusion, the findings from this thesis suggest that cryotherapy offers little benefit as an acute recovery strategy following strenuous exercise, and in contrast to much of the anecdotal evidence, WBC offers no enhanced recovery benefit compared to CWI. Further, whilst the use of habitual CWI exposure may enhance neural adaptations to training, this benefit is likely to be offset by reductions in longer term hypertrophic gains and attenuated improvements in perceptual recovery during a resistance training programme compared to a placebo intervention. Further research with different athlete populations and alternative sport specific markers is

warranted to gain a clearer overall picture of the possible role of cryotherapy in a sporting context.

7.8. Practical recommendations

Based on the findings from the studies within this thesis, there is little evidence to suggest that cryotherapy is an effective intervention for the recovery of muscle function post exercise. Whilst cryotherapy is potentially useful for the reduction of muscle soreness following an acute exercise session, habitual exposure may be disadvantageous. In regards to the potentially enhanced benefits of WBC compared to CWI, the evidence presented here does not support this notion. As such, where athletes and practitioners choose to continue using cryotherapy, CWI remains the most cost effective and accessible option.

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Appendices

Appendix 1

Middlesex University
London Sport Institute

School of Science and Technology
The Burroughs
London NW4 4BT

www.mdx.ac.uk
Main switchboard:
020 8411 5000

To: Katherine Paice, Laura Wilson, Scott Sinclair, Shen Palihavadana, & Tanwir Faki

Date: 05 May 2015

Dear Katherine Paice, Laura Wilson, Scott Sinclair, Shen Palihavadana & Tanwir Faki

RE: Application 357 "Effects of cryotherapy and/or cherry juice supplementation on markers of muscle damage, pulmonary inflammation, upper respiratory tract symptoms and recovery following completion of a 26.2 mile running race." Supervisor: Dr Lygeri Dimitriou Category: A2, A4, A5, A6 & A7

Thank you for the response which adequately answers the ethics committee's queries. On behalf of the London Sport Institute Ethics Subcommittee, I am pleased to give your project its final approval. Please note that the committee must be informed if any changes in the protocol need to be made at any stage.

Yours sincerely

Dr. Rhonda Cohen
Chair of Ethics Sub-committee (London Sport Institute)

Middlesex University
London Sport Institute

School of Science and Technology
The Burroughs
London NW4 4BT

www.mdx.ac.uk
Main switchboard:
020 8411 5000

To: Laura Wilson

Date: 26 July 2016

Dear Laura

RE: Application 548 "The effectiveness of cold water immersion and whole body cryotherapy on markers of recovery following strenuous resistance exercise" Supervisor Dr Emma Cockburn
Category: A5 & A7

Thank you for the response which adequately answers the ethics committee's queries. On behalf of the London Sport Institute Ethics Subcommittee, I am pleased to give your project its final approval.

Please note that the committee must be informed if any changes in the protocol need to be made at any stage.

Yours sincerely

Dr. Rhonda Cohen
Chair of Ethics Sub-committee (London Sport Institute)

Middlesex University
London Sport Institute

School of Science and Technology
The Burroughs
London NW4 4BT

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Main switchboard:
020 8411 5000

To: Laura Wilson, Patricia Baez, Gutieerez, Bilaal Syed, Eleftheria Panagiotou, and Vlad Turek
Co-investigators: Anthony Turner, Stuart Miller, Lygeri Dimitriou, Shavda Chavda and Chris Bishop

Date: 10 May 2016

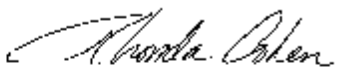
Dear All

RE: Application 547 "The effect of cold water immersion and whole body cryotherapy on physiological adaptations and muscle performance following 8 weeks strength and power training." Supervisor Dr Emma Cockburn Category: A5 & A7

Thank you for the response which adequately answers the ethics committee's queries. On behalf of the London Sport Institute Ethics Subcommittee, I am pleased to give your project its final approval.

Please note that the committee must be informed if any changes in the protocol need to be made at any stage.

Yours sincerely



Dr. Rhonda Cohen
Chair of Ethics Sub-committee (London Sport Institute)

Appendix 2

MIDDLESEX UNIVERSITY
SCHOOL OF SCIENCE AND TECHNOLOGY

LONDON SPORT INSTITUTE ETHICS SUB-COMMITTEE

PARTICIPANT INFORMATION SHEET (PIS)

Participant ID Code:……

1. Study title

"Effects of cryotherapy and/or cherry juice supplementation on markers of muscle damage, pulmonary inflammation, upper respiratory tract symptoms and recovery following completion of a 26.2 mile running race"

2. Invitation paragraph

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

3. What is the purpose of the study?

This study is being undertaken as part of a postgraduate research project within the School of Science and Technology at Middlesex University. Muscle damage following intensive exercise is very common and can be characterised by soreness, inflammation, strength loss and changes in blood borne markers. Additionally, symptoms and signs of pulmonary and airway inflammation (e.g., cough; coloured discharge; sore throat; watery eyes; nasal symptoms, wheezing, shortness of breath and other) following prolonged and exhaustive exercise have been repeatedly reported. It is possible that this might be increasing the likelihood of viral infection and developing airway hyperreactivity. Identifying recovery and/or supplementation strategies that can alleviate symptoms of muscle damage and/or pulmonary inflammation or speed up recovery following exercise is becoming increasingly important to athletes and practitioners. Therefore, the purpose of this study is to investigate the effectiveness of 4 different recovery strategies on markers of muscle damage, recovery, systemic and pulmonary inflammation following completion of a 26.2 mile running race.

4. Why have I been chosen?

It is important that we assess as many participants as possible, and you have indicated that you are interested in taking part in this study. This study is seeking to investigate the effects of specific recovery strategies on recovery in recreationally active adult males aged between 18 and 55 years. Participants must be in good general health and will be required to have previously completed a 26.2 mile running race and have an expected completion time of 4 and a half hours or less. Unfortunately you will be unable to participate if you suffer from any contra-indications to the study, including; lower limb injuries, Raynaud's syndrome, sensitivity to the cold, circulatory problems, respiratory problems such as asthma or COPD, smoker, recent infection or if you suffer from needle phobia. We would also ask that you abstain from taking protein, BCAA or creatine supplements or anti-inflammatory medication (e.g. ibuprofen), or using other therapeutic therapies such as massage, heat packs or acupuncture for the duration of the study. We would also ask that you refrain from strenuous exercise for at least 72 hours before any of the testing sessions and for the duration of the study other than completion of the 26.2 mile running race itself. We hope to recruit a minimum of 75 participants.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you do decide to withdraw from the study then please inform the researcher as soon as possible, and they will facilitate your withdrawal. If, for any reason, you wish to withdraw your data please contact the researcher within a month of your participation. After this date it may not be possible to withdraw your individual data as the results may have already been published. However, as all data are anonymised, your individual data will not be identifiable in any way.

A decision to withdraw at any time, or a decision not to take part, will not affect your place in future races or studies.

6. What will I have to do?

The study will involve you attending the Middlesex University laboratories at Allianz Park for at least 3 consecutive days (ideally 4).

Questionnaires

Daily Analysis of Life Demands for Athletes (DALDA)

You will be asked to complete a DALDA questionnaire on each visit to the laboratory. The DALDA can be used to measure changes in stress and recovery states in athletes. The DALDA is a 2 part questionnaire that aims to identify both sources and symptoms of stress. Part A identifies potential sources of stress (including home life, work, sleep and sports training), whilst part B allows you to rate symptoms of stress and fatigue as worse than normal, normal, or better than normal.

Sleep Diary
You will be asked to complete a simple sleep quality questionnaire every night for one week before and 2 weeks after the race. This consists of a sleep diary (monitoring time taken to fall asleep, hours spent asleep, etc) and a quality questionnaire (assessing mental alertness, tension, wellness and enjoyment).

Incidence and severity of upper respiratory tract symptoms (URTS) and illness

You will be asked to report for 21 days (7 days before and 14 after the race) any incidence of: 1) cold symptoms (e.g., cough, sore throat); 2) flu symptoms (e.g., fever, headache, general aches and pains, fatigue); 3) nausea symptoms (vomiting, and/or diarrhoea); 4) muscle, joint, or bone problems/injury; 5) allergy symptoms; 6) other health problems; and rate their severity.

- **Muscle Soreness**
  On each visit to the laboratory, you will be asked to indicate perceived levels of muscle soreness of your quadriceps and hamstrings using a numbered scale (visual analogue scale). The scale will be numbered from 0 to 10, where 0 indicates no soreness on movement, and 10 indicates that the muscles are too sore to move. You will be asked to rate your perceived level of soreness at rest and during a squatting movement. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Blood markers**
  Whole blood samples (approx. 15ml, 3 tablespoons) will be collected from a vein in your arm for the purpose of assessing muscle damage, inflammation and oxidative stress, the day. The procedure will be the same as a routine blood test. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Allergy test determination**
  A very small blood sample (one blood drop) will be collected from your fingertip or earlobe on a capillary tube to assess whether you are allergic to 20 different triggering substances, covering over 90% of the most common allergies in Europe. This test will be performed on your initial visit which will act as a habituation session.

- **Induced sputum**
  You will be asked to use a nebulizer with a saline solution for 12 min. At 2-min intervals, you will be asked to clear saliva from your mouth and then cough or spit to collect fluid from your lungs. The sputum will be collected in a sterile plastic container for further processing. Contamination of sputum with saliva is major concern and problem therefore you will be asked to wear nose clips or wash your mouth. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

  Lung function
  Lung function (forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow rate (PEFR) will be measured by spirometry. You will be asked to breathe in as much air as possible, and then blow out as hard as you can, into a mouth piece connected to a spirometer until your lungs feel completely empty for three times. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Marker of Lung Inflammation: Exhaled and nasal Nitric Oxide (NO)**
  You will be asked to inhale sharply and then exhale at a steady rate so NO can be measured. Nasal NO will be sampled directly from both nostrils and will be reported as the mean of six measurements. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Muscle function**
  You will be strapped into a machine in a seated position with the knee of your dominant leg flexed at 90 degrees. After assessing your range of movement you will be asked to complete flexion and extension of your knee at 60 $deg \cdot s^{-1}$. After 2 warm up trials you will complete 3 maximal efforts. You will also be asked to complete a maximal voluntary isometric contraction. To do this your knee will be fixed at 90 degrees and you will be asked to push against an immovable arm. After 2 warm up trials you will complete 3 maximal efforts. From this measurement we will also calculate rate of force development. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Drop jump**

You will be instructed to drop from a platform at a height of 50cm onto the floor and then jump vertically for maximum height as quickly as possible. You will be required to keep your hands on your hips for the duration of the movement. Height, flight time and ground contact time/reaction time will be recorded for each jump using a force plate interfaced with a laptop. Each jump will be attempted 3 times at each testing session. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

- **Counter movement jump**
  You will stand in a relaxed upright position with your feet shoulder width apart, hands on your hips and knees fully extended. You will then make a downward countermovement to a squat position (knee joint angle of 90º) before jumping vertically for maximal height. The jump must be performed in one continuous movement with hands remaining on hips for the duration. Height, flight time and ground contact time/reaction time will be recorded for each jump using a force plate interfaced with a laptop. Each jump will be attempted 3 times at each testing session. This test will be performed 5 times (immediately before, immediately post, 24 h, 48 h and 72 h following completion of the 26.2 mile race).

On the first visit to the lab you will complete the 26.2 mile running race. The race will take place on a pre-determined route surrounding the field grounds of Allianz Park stadium. Following completion of the race, you will be asked to return to the lab to complete your allocated recovery intervention. Following completion of your allocated recovery intervention you will be expected to repeat the tests as listed above. This visit will take approximately 6 hours but will depend on how long it takes to complete the race. You will be allocated to one of 5 groups as detailed below (you will only complete one of the interventions);

Whole Body Cryotherapy (WBC)
You will be exposed to two cold treatments in a cryotherapy chamber. The first exposure will last 3 minutes and the temperature of the main chamber will be maintained at -110 degrees (+/- 5º). You will be asked to walk around slowly whilst flexing and extending your elbows and fingers throughout the 3 minutes. You will then have a 15 minute warming period in an ambient room before entering the chamber for a further 4 minute bout. Whilst in the chamber you will be asked to remove glasses, contact lenses and any jewellery or piercings. During exposure, you will wear a pair of shorts and nothing above the waist. You will also wear gloves, dry socks and shoes, a headband covering the ears and a mask to protect the nose and mouth.

Cold Water Immersion (CWI)
You will sit in a mobile ice bath (iSprint/iBody) ensuring your lower limbs and hips are fully immersed. You will remain in the ice bath filled with water cooled to 8 degrees (+/- 0.5º) for 10 minutes. The ice bath will be connected to a chiller unit so that the water temperature can be monitored and maintained within the desired parameters for the duration of the treatment. Immediately after CWI you will be asked to towel yourself dry and change into clean, dry clothing.

Cherry Juice (CJ)
You will be asked to take the supplement for five days prior to, the day of the 26.2 mile race and for the 48 h following the race (total eight days). Following completion of the race, you will be asked to sit quietly in a temperature regulated lab for 10 minutes.

Control
Participants in the control group will be asked to take a placebo fruit juice drink for 5 days prior to, the day of and 2 days after the race (total eight days). Participants in the control group will be asked to rest quietly in a temperature controlled lab for 10 minutes following completion of the 26.2 mile race.

Cocktail
Participants in the cocktail group will complete both the cherry juice and CWI interventions as outlined above.

Due to the nature of the intervention it will not be possible to blind participants to their experimental treatment. Participants will receive no information regarding the expected outcomes of each intervention in an effort to minimise any bias.

Following completion of the intervention, all participants will be asked to repeat all tests completed immediately before the race except for the allergy test.

Please note that in order to ensure quality assurance and equity this project may be selected for audit by a designated member of the committee. This means that the designated member can request to see signed consent forms. However, if this is the case your signed consent form will only be accessed by the designated auditor or member of the audit team.

7. Will I have to provide any bodily samples (i.e. blood/saliva/urine)?
We will need to take a blood test from you on each of your visits as specified above. This will only be carried out by trained members of the team and will be tested within Middlesex University. Should the tests reveal an abnormality (were recognised clinical guidelines regarding test results exist) the researcher will recommend to you that you seek further medical advice from your GP. Bear in mind though that a single test may not always provide an accurate reflection of your health status.

8. What are the possible disadvantages and risks of taking part?

All participants may experience some muscle soreness after the race but this is completely normal and should subside within a few days. There will be a sharp scratch from the needle during the blood tests and some possible bruising. Participants might experience some mild discomfort as a result of the lung and muscle function tests but this will subside and will not have any lasting effect. Participants who are in cryotherapy groups may experience some mild discomfort from the cold temperatures in the ice bath or cryotherapy chamber. It should be noted that these techniques are commonly used in research and are safe.

There will be no psychological discomfort.

Appropriate risk assessments for all procedures have been conducted, and will be followed throughout the duration of the study.

9. What are the possible benefits of taking part?

We hope that participating in the study will help you. However, this cannot be guaranteed. All participants will be offered free physiological (determination of running economy, lactate threshold and VO2 max) and total body composition testing following their involvement in the study. This testing uses state of the art equipment and would usually cost upwards of £500. It will all be carried out by trained members of the team and you will be provided with your results to take home. This is a fantastic opportunity to experience this testing and improve your running performance. You will not be provided with your results until you have completed the study.

The information we get from this study may help us to understand potential benefits of different recovery or supplementation strategies on recovery from exercise and suppression of inflammation and upper respiratory tract symptoms following completion of a 26.2 mile running race.

9. Will my taking part in this study be kept confidential?

The research team has put a number of procedures in place to protect the confidentiality of participants. You will be allocated a participant code that will always be used to identify any data you provide. Your name or other personal details will not be associated with your data, for example, the consent form that you sign will be kept separate from your data. All paper records will be stored in a locked filing cabinet, accessible only to the research team, and all electronic data will be stored on a password protected computer. All information you provide will be treated in accordance with the UK Data Protection Act.

10. What will happen to the results of the research study?

The results of the research study will be used as part of a Postgraduate dissertation. The results may also be presented at conferences or in journal articles. However, the data will only be used by members of the research team and at no point will your personal information or data be revealed.

11. Who has reviewed the study?

The study has received full ethical clearance from the Research ethics committee who reviewed the study. The committee is the Middlesex

University, School of Science and Technology, London Sport Institute Ethics sub-committee

12. Contact for further information

If you require further information, have any questions or would like to withdraw your data then please contact:

mdxmarathon@gmail.com

Student researchers: Laura Wilson, Kat Paice, Scott Sinclair and Tanwir Faki
Supervisors: Lygeri Dimitriou (l.dimitriou@mdx.ac.uk) and Emma Cockburn (e.cockburn@mdx.ac.uk)

Thank you for taking part in this study. You should keep this participant information sheet as it contains your participant code, important information and the research teams contact details.

Appendix 3

CONSENT FORM

Title of Project: The effectiveness of cold water immersion, whole body cryotherapy and branched chain amino acids (BCAA's) on markers of recovery following strenuous resistance exercise.

Name of Researcher: Laura Wilson

1. I confirm that I have read and understood the Participant Information Sheet for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

3. I agree that this form that bears my name and signature may be seen by a designated auditor.

4. I agree that my non-identifiable research data may be stored in National Archives and be used anonymously by others for future research. I am assured that the confidentiality of my data will be upheld through the removal of any personal identifiers.

5. I understand that sections of any of my medical notes may be looked at by responsible individuals from Middlesex University or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

6. I agree to take part in the above study.

Name of participant	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature
Name of parent/guardian (if appropriate)	Date	Signature

1 copy for participant; 1 copy for researcher.

Appendix 4

HEALTH QUESTIONNAIRE

Name: ……
Date of Birth: ……
Participant number: ……

As you are participating in exercise within this laboratory, please can you complete the following questionnaire. Your co-operation is greatly appreciated. All information within this questionnaire is considered confidential.

Where appropriate please circle your selected answer.

1. How would you describe your current level of activity?
   Sedentary / Moderately Active / Highly Active

2. How would you describe your current level of fitness?
   Very Unfit / Moderately Fit / Trained / Highly Trained

3. How would you describe your current body weight?
   Underweight / Ideal / Slightly Overweight / Very Overweight

4. Smoking Habit: -
   Currently a non-smoker Yes / No
   Previous smoker Yes / No
   If previous smoker, how long since you stopped? ……Years
   Regular smoker Yes / No of …… per day
   Occasional smoker Yes / No of …… per day

5. Alcohol Consumption: -
   Do you drink alcohol? Yes / No
   If yes then do you - have an occasional drink Yes / No
   Have a drink every day? Yes / No
   Have more than one drink per day? Yes / No

6. Have you consulted your doctor within the last 6 months?
   Yes / No
   If yes, please give details to the test supervisor

7. Are you currently taking any medication (including anti-inflammatory drugs)?
   Yes / No
   If yes, please give details to the test supervisor

8. Do you, or have you ever suffered from:-
   Diabetes Yes / No
   Asthma Yes / No
   Epilepsy Yes / No

Bronchitis Yes / No
Elevated cholesterol Yes / No
High Blood Pressure Yes / No
Blood borne disease or infection Yes / No

9. Do you suffer from, or have you ever suffered from any heart complaint or pains in your chest, either associated with exercise or otherwise?
   Yes / No

10. Is there a history of heart disease in your family?
    Yes / No

11. Do you feel faint or have spells of severe dizziness when undertaking exercise or otherwise?
    Yes / No

12. Do you currently have any form of muscle joint injury?
    Yes / No

13. Have you ever suffered from any knee joint injury or thigh injury?
    Yes / No

14. Have you had any reason to suspend your training in the last 2 weeks?
    Yes / No

15. Do you currently take any anti-inflammatory medication?
    Yes / No
    If yes, please give details to the test supervisor

16. Do you currently take any form of nutritional supplement (e.g. creatine, whey and casein protein, HMB, anti-oxidants, etc)?
    Yes / No
    If yes, please give details to the test supervisor

17. Do you currently take, or have you ever taken steroids?
    Yes/No
    If yes, please give details to the test supervisor

18. Do you have a medical condition that is exacerbated by exposure to cold temperatures (e.g. Raynaud's syndrome, chilblains, etc)?
    Yes / No
    If yes, please give details to the test supervisor

19. Do you suffer from needle phobia or haemophilia?
    Yes / No

20. Is there anything to your knowledge that may prevent you from successfully completing the tests that has been explained to you?

Yes / No
If yes, please give details to the test supervisor

Please provide any further information concerning any condition/complaint that you suffer from and any medication that you may be taking by prescription or otherwise.

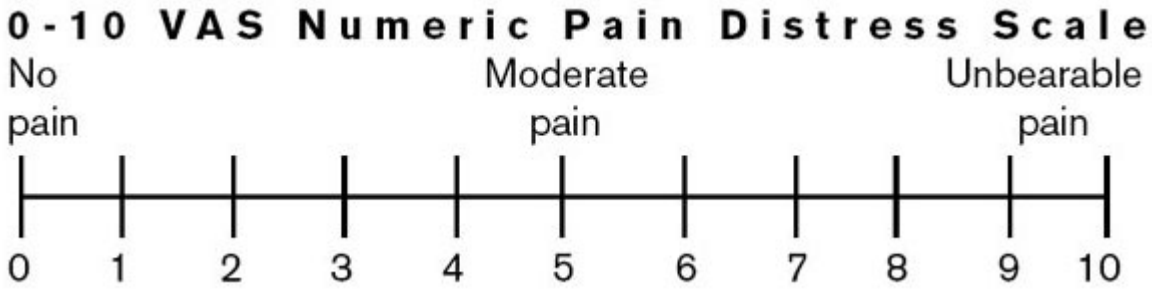
……
……

Signature of Participant: ……

Signature of Researcher: ……

Date: ……

Appendix 5


0-10 VAS Numeric Pain Distress Scale
No pain
Moderate pain
Unbearable pain
0 1 2 3 4 5 6 7 8 9 10

Appendix 6

DALDA Questionnaire

Part A

	Worse than normal	Normal	Better than normal
Diet			
Home Life			
School/College/Work			
Friends			
Training and Exercise			
Climate			
Sleep			
Recreation			
Health			

Part B

	Worse than normal	Normal	Better than normal
Muscle Pains			
Techniques			
Tiredness			
Need for a rest			
Supplementary Work			
Boredom			
Recovery Time			
Irritability			
Weight			
Throat			
Internal			
Unexplained Aches			
Technique Power			
Enough Sleep			
Between Sessions Recovery			
General Weakness			
Interest			
Arguments			
Skin Rashes			
Congestion			
Training Effort			
Temper			
Swellings			
Likeability			
Running Nose			

TABLE 2. DEFINITIONS OF PART B (SYMPTOMS OF STRESS) FOR THE DAILY ANALYSES OF LIFE DEMANDS FOR ATHLETES.

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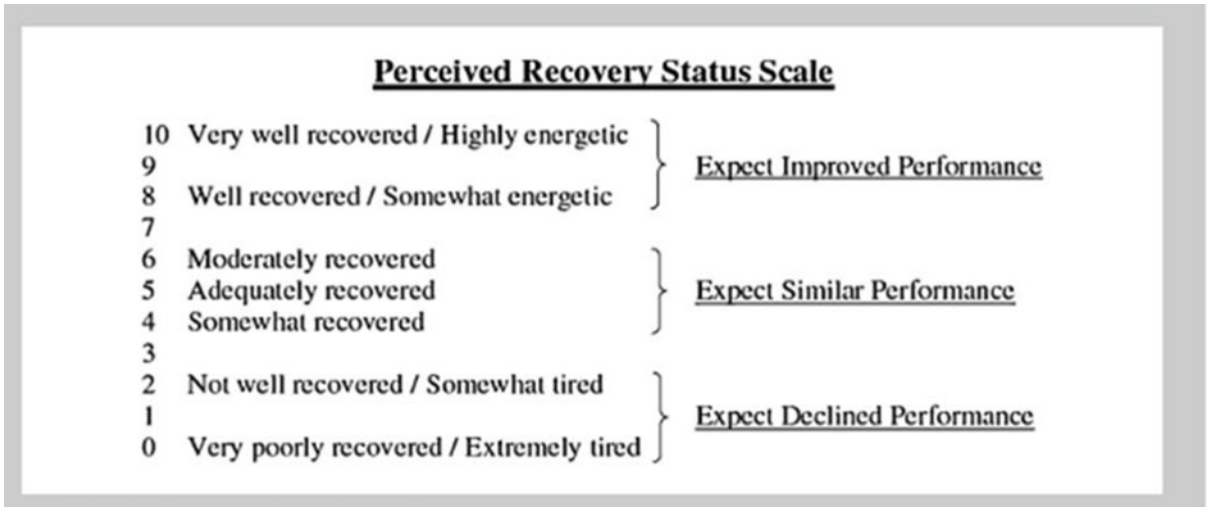
1. Muscle pains. Do you have sore joints and/or pains in your muscles?
2. Techniques. How do your techniques seem/feel to you? Have your technical skills changed?
3. Tiredness. Your general state of tiredness is:
4. Need for a rest. Do you feel that you need a rest between training sessions?
5. Supplementary work. How strong do you feel when you do supplementary training (e.g., weights, resistance work, stretching)?
6. Boredom. How boring is training?
7. Recovery time. Do the recovery times between each training effort need to be longer?
8. Irritability. Are you irritable? Do things get on your nerves?
9. Weight. How is your weight?
10. Throat. Have you noticed your throat being sore or irritated?
11. Internal. How do you feel internally? Have you had constipation, upset stomachs, etc.?
12. Unexplained aches. Do you have any unexplained aches or pains?
13. Technique power. How do you rate the level of power you develop in your techniques?
14. Enough sleep. Are you getting enough sleep?
15. Between sessions recovery. Are you tired before you start your second training session of the day?
16. General weakness. Do you feel weak all over?
17. Interest. Do you feel that you are maintaining your interest in your sport?
18. Arguments. Are you having squabbles and arguments with people?
19. Skin rashes. Do you have any unexplained skin rashes or irritations?
20. Congestion. Are you experiencing congestion in the nose and/or sinuses?
21. Training effort. Do you feel that you can give your best effort at training?
22. Temper. Do you lose your temper?
23. Swellings. Do you have any lymph gland swellings under your arms, below your ears, in your groin, etc.?
24. Likability. Do people seem to like you?
25. Running nose. Do you have a running nose?

Appendix 7


Perceived Recovery Status Scale
10 Very well recovered / Highly energetic
9
8 Well recovered / Somewhat energetic
7
6 Moderately recovered
5 Adequately recovered
4 Somewhat recovered
3
2 Not well recovered / Somewhat tired
1
0 Very poorly recovered / Extremely tired
Expect Improved Performance
Expect Similar Performance
Expect Declined Performance

Appendix 8

Participant ID……

Date:					
What time did you go to bed last night?					
What time did you get out of bed this morning?					
How long did it take you to fall asleep?					
How many times were you awake in the night?					
How was the quality of your sleep? 1 = very good 5 = very poor					

Instructions:

1. Please insert the date (e.g. Monday 10th July) for that evenings sleep
2. Please state the time you went to bed (not went to sleep), e.g. 22:04
3. Please state the time you got out of bed (not when you woke up) e.g. 07:43
4. Please state how many minutes it took you to fall asleep after you went to bed, e.g. 15 minutes
5. Please state the number of times you woke up once you had fallen asleep
6. Please rate on a 1 to 5 scale the quality of your sleep (1 = very good; 2 = good; 3 = neutral; 4 = poor; 5 = very poor)