Title: The effects of contrast water therapy and hot water immersion on the signs and symptoms of exercise-induced muscle damage following a downhill run

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The effects of contrast water therapy and hot water immersion on the signs and symptoms of exercise-induced muscle damage following a downhill run

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By

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Declaration

I declare that this thesis is my own work. It is being submitted for the degree of MSc by Research at the University of Bedfordshire.
It has not been submitted for any degree or examination in any other University or educational institute

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'*It always seems impossible until it's done. – Nelson Mandela*

I dedicate this to Daddy. Forever gone, never forgotten

List of abbreviations, acronyms and symbols

ADP	adenine diphosphate
ATP	adenine triphosphate
BF	bicep femoris
cm	Centimetre
CK	creatine kinase
CMJ	countermovement Jump
^{0}	Degrees
^{0}C	degrees Celsius
DOMS	delayed onset muscle soreness
DHR	downhill run
ECC	eccentric exercise
EF	elbow flexors
EE	elbow extensors
EIMD	Exercise-induced muscle damage
Flex	flexibility
F	Female
GN	gastrocnemius
h	Hour

IU/L	international units per litre
ISO	isometric contraction
L	left leg
KE	knee extensors
KF	knee flexors
LC	limb circumference
M	Male
MVC	maximal voluntary contraction
min	Minute
mm	Millimetres
PPT	pressure pain threshold
Pre	before exercise
Post	after exercise
R	right leg
RF	rectus femoris
ROM	range of motion
RPE	rate of perceived exertion
SSC	stretch-shortening cycle
SJ	squat jump

T_{skin}	skin temperature
VAS	visual analogue scale
$\dot{V}O_{2max}$	maximal oxygen consumption
vVO_{2max}	Velocity at $\dot{V}O_{2max}$
%	Percentage

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Abstract

Eccentric exercise typically induces muscle damage that can cause detrimental effects on an athlete's performance. Therefore, it is vital to find a recovery strategy that will increase the rate of recovery, alleviate the signs and symptoms associated with exercise-induced muscle damage, and return the athlete to peak performance levels as quickly as possible. Water immersion, in the form of cold water immersion, hot water immersion and contrast water therapy are becoming increasingly popular interventions used to alleviate the signs and symptoms of exercise-induced muscle damage and improve recovery after eccentric exercise.

The purpose of the study was to determine the effects of contrast water therapy and hot water immersion on the signs and symptoms of exercise-induced muscle damage using indirect markers of muscle damage. These markers of muscle damage include; perceived muscle soreness, pressure pain threshold, squat jump, range of motion, flexibility, creatine kinase and limb circumference and are used in this study as a measure of the signs and symptoms of exercise-induced muscle damage.

The purpose of Chapter 4 (Study 1) was to determine the reproducibility of these indirect markers of muscle damage. Overall, no systematic bias was found for any of the variables apart from creatine kinase. However, several performance and functional measurement tools; creatine kinase, flexibility, perceived soreness and pressure pain threshold exhibited low reproducibility, whilst creatine kinase, range of motion, perceived muscle soreness, limb circumference, and pressure pain threshold demonstrated good to excellent reliability however, squat jump demonstrated good

reliability. With several of the indirect markers of muscle damage demonstrating poor to low reliability, discretion is advised when using these measures to detect systematic change, as the low reproducibility may obscure the true experimental results.

The purpose of Chapter 5 (Study 2) was to determine the effects of contrast water therapy, and hot water immersion on the signs and symptoms of exercise-induced muscle damage. There is a plethora of studies aimed at determining the effects of cold water immersion and contrast water therapy on the signs and symptoms of exercise-induced muscle damage (Bailey et al., 2007, Eston and Peter., 1999a, Halson et al., 2008., Ingram et al., 2009, Montgomery et al., 2008, Vaile et al., 2008b). However, limited studies have determined the effects of hot water immersion alone (Kuligowski et al., 1998). All participants in this study completed a muscle damaging protocol which consisted of a 40 minute downhill run. Subsequently, participants were either treated with hot water immersion, contrast water therapy, or no water immersion for 40 minutes at 0 hours, 24 hours, and 48 hours post downhill run. Perceived muscle soreness, pressure pain threshold, squat jump, range of motion, flexibility, limb circumference and creatine kinase were measured on five separate occasions (pre-downhill run, immediately post downhill run, 24, 48 and 72 hours post downhill run). Results demonstrated that contrast water therapy had an improved effect on alleviating several signs and symptoms of exercise-induced muscle damage. Briefly, peak decrements at 24 hours in creatine kinase, pressure pain threshold, flexibility, squat jump, and perceived muscle soreness were found in the hot water immersion group and contrast water immersion groups, where as peak decrements at 48 hours for pressure pain threshold, flexibility, and squat jump were found in the control group.

Key words: reproducibility, water immersion, contrast water therapy, cold water immersion, hot water immersion, exercise-induced muscle damage, eccentric exercise

Chapter 1:

General Introduction

1.1 Introduction

During long intensive training periods and strenuous competition phases athletes place a large amount of physiological stress on their bodies (Poppendieck et al., 2013). Additionally, resistance exercises, which may incorporate eccentric contractions (ECC), are a popular component of an athlete's training regime (Howatson, 2008), as it has been shown that eccentric loading is beneficial for increasing muscle size and strength (Nosaka & Newton, 2002). Although ECC have been shown to induce greater hypertrophy when compared with intensity matched concentric contractions (Howatson, 2008, Newham et al., 1983), ECC predominately leads to exercise-induced muscle damage (EIMD) (Leeder et al., 2012). The signs and symptoms of EIMD include a decrease in neuromuscular function (Highton et al., 2009, Howatson, 2008), swelling and structural damage (Cleak and Eston, 1992b, Eston et al., 2003), delayed onset muscle soreness (DOMS) (Raastad et al., 2010), decrease in range of motion (Eston and Peters, 1999a, Clarkson and Newham, 1994), an increase in limb circumference measurements (Friden et al., 1988), and an increase in circulating blood proteins (Clarkson and Hubal., 2002). Furthermore, these signs and symptoms are most prominent when performed in an unaccustomed condition (Armstrong et al., 1991 & Clarkson et al., 1992). Additionally, the signs and symptoms of EIMD have been shown to peak 24 – 48 h post muscle damaging exercise (Eston et al., 1996) and may persist for up to 7 days (Armstrong, 1984, Friden et al., 1983) or more (Faulkner et al., 1993). Subsequently, this may negatively impact an athlete's ability to perform maximally and sub-maximally, especially when repeated performance is required in tournament scenarios and/or multiple-day competitions (Leeder et al., 2012). Therefore, it is essential that researchers investigate appropriate recovery strategies to

alleviate the signs and symptoms associated with EIMD in order to improve recovery, and attenuate subsequent performance decrements.

The 'typical' indirect markers of EIMD include, but not limited to; range of motion (ROM), limb circumference, squat jump, increased muscle soreness, flexibility, pressure pain threshold (PPT), and creatine kinase (CK) activity. Overall, there is an extensive debate in the literature concerning the reliability and the reproducibility of these indirect markers of EIMD, with several studies demonstrating different values for reliability and reproducibility showing conflicting findings and studies not being well documented. Therefore, it is essential that during this study, the reliability and reproducibility of each indirect marker is found using several different methods in order to obtain a comparison between different markers and different studies.

There is a plethora of recovery strategies that have been shown to attenuate EIMD and DOMS and improve recovery. These strategies include, but are not limited to: compression garments (Hill et al., 2013, Houghton et al., 2009, Jakeman et al., 2010), myofascial release (MacDonald et al., 2013, Sullivan et al., 2013), nutritional strategies, for example; milk (Cockburn et al., 2013, Shaw et al., 2013), cherry juice (Howatson et al., 2010, Bowtell et al., 2011, Connolly et al., 2006) and branch chain amino acids (Howatson et al., 2012). External cooling methods, for example; cold water immersion (Ascensao et al., 2011, Eston and Peters, 1999a, Goodall and Howatson, 2008, Gregson et al., 2011), ice vests (Barr et al., 2011, DeMartini et al., 2011), ice packs (McDermott et al., 2009), cryotherapy (Hausswirth et al., 2011), ice massage (Isabell et al., 1992), hot water immersion (Kuligowski et al., 1998) and contrast water therapy (Higgins et al., 2011, Coffey et al., 2004) have been shown to

be effective in alleviating perception of pain and muscle damage, and improving performance after EIMD (Leeder et al., 2012).

Water immersion has been shown to reduce the signs and symptoms associated with EIMD following muscle damaging exercise (Calder, 2003, Wilcock et al., 2006a), due to the decrease in swelling and analgesic effects of cold water (Cheung et al., 2003). However, the optimal timing, temperature(s), and duration of water immersion required to attenuate the negative signs and symptoms of EIMD remains unclear. Furthermore, there is wide variation in the protocols used within the literature which makes the comparison of studies problematic. Cold water immersion (CWI) (5^0C - 15^0C) is increasingly becoming a popular water immersion strategy (Venter, 2014). Nevertheless, immersing an athlete or individual in cold water for an extended period of time may be uncomfortable or intolerable. Alternatively, hot water immersion (HWI) (38^0C - 39^0C) may be more tolerable, and equally as valid (Peiffer et al., 2009) as CWI. Furthermore, contrast water therapy (CWT), in which water immersion is alternated between cold water and hot water is also increasingly becoming utilised as a method of recovery (Cochrane, 2004) and therefore, will be included in this study. This alternation between cold water and hot water may bring about the same benefits of being immersed in cold water however, may be more tolerable. Recovery strategies also have to be cost effective, functional and easily adaptable in the field. Subsequently, further research is required in order to establish the optimal water immersion protocol required to alleviate the signs and symptoms of EIMD as this may improve competition performance and allow greater training loads (Versey et al., 2013).

1.2 Aims

The aim of this thesis is to determine whether CWT and HWI can alleviate the signs and symptoms of EIMD following a 40 min downhill run.

1.3 Research Questions and Experimental Hypotheses

The aims will be achieved through the following objectives:

Study 1: *To investigate the reproducibility and the reliability of the measurements tool used in this study to assess the signs and symptoms of EIMD.*

Study 2: *To investigate the effectiveness of CWT and HWI in alleviating the signs and symptoms associated with muscle damaging exercise.*

It is hypothesised that CWT and HWI will have a beneficial effect on the signs and symptoms of EIMD, with the greater attenuation demonstrated in the CWT group.

Chapter 2:

Literature Review

2.1 Overview

This literature review will be separated into two main sections. Section 1 will discuss the mechanisms of ECC and EIMD, which will further be split into two sub sections discussing the initial phase and secondary phase of EIMD. Subsequently, the phenomenon of DOMS will also be discussed, and the effect that EIMD will have on the functional and performance measures used to measure the effects of EIMD. The second section of the literature review will focus on the effects of CWT and HWI on the signs and symptoms of EIMD.

2.2 Section 1: Eccentric Exercise and the Development of Exercise-Induced Muscle Damage

Eccentric contractions occur when the external force acting on a muscle is greater than the force being exerted by the muscle itself (Howatson, 2008, Newham et al., 1986, Newham et al., 1983, Armstrong et al., 1983, MacIntyre et al., 1996, Brown et al., 2010) causing the active muscle to be lengthened forcefully (Proske and Morgan, 2001). The force developed during an eccentric contraction is approximately two-fold greater than that developed during a concentric muscle contraction (Faulkner et al., 1993, MacIntyre et al., 1996, Raastad et al., 2010). Furthermore, eccentric muscle contractions have been shown to preferentially recruit more type II fibres (Zhou et al., 2011) with fewer motor units being recruited. When an eccentric contraction is performed, it is suggested that during the active stretch of the muscle, the length change will be taken up by the weakest sarcomeres in the myofibrils or alternatively the weakest half sarcomeres (Morgan, 1990, Proske & Morgan, 2001). These sarcomeres will lengthen rapidly to a point of no myofilament overlap causing these sarcomeres to become progressively weaker (Proske & Morgan, 2001). This causes

profound skeletal muscle structural and functional damage (Davies et al., 2011b) resulting in changes of both voluntary and electrically stimulated force generation in skeletal muscle compared to intensity matched concentric contractions (Newham et al., 1983) due to the ultrastructural damage to the muscle fibres.

When a load is lifted and a concentric contraction is performed, a hypothetical 3,000 motor-units will be activated, when the same load is lowered during an eccentric contraction, for reasons not understood (Armstrong et al., 1984, Newham et al., 1983, Evans & Cannon, 1991), only a third to a sixth of these motor-units will be activated. Since the weight is the same, more stress is placed on the fewer muscle fibres during the eccentric contraction (Smith et al., 1992). This stress results in temporary, sub cellular disruptions in the tissue involved. The initial sub cellular disruption occurs during the exercise and may be seen immediately after termination of exercise, and the extent of this micro injury will increase during the next 48 h (Armstrong et al., 1983, Newham et al, 1983), even without further performance of an exercise that would cause more damage (Smith et al., 1992). This reduction in the amount of muscle fibres used could be due to the reduced metabolic rate coupled with enhanced tension generation in comparison to concentric contractions (Newham et al, 1893). Therefore, a small cross-sectional area of muscle is activated to handle the same load (Clarkson and Sayers, 1999). Consequently, structural damage to the skeletal muscle occurs, which may present as myofibrillar disturbance and Z-line damage (Friden et al., 1983).

Complete recovery and restoration of muscle function following ECC may take a week or longer (Clarkson & Tremblay, 1988, Friden et al., 1983, Newton et al., 1987). Friden et al., (1983) found evidence of ultrastructural damage in biopsies taken

immediately and three days after subjects performed eccentric cycle ergometer exercise. By six days post exercise, disturbances were still evident in 12% of the observed fibres. Furthermore, Clarkson & Tremblay, (1988) monitored DOMS, range of motion (ROM), and creatine kinase (CK) following ECC and found no measure had returned to baseline within five days post exercise. This evidence suggests that recovery from ECC is a relatively slow process (Ebbelling & Clarkson, 1990).

Downhill running (DHR) is considered to be predominantly eccentric (Proske and Morgan, 2001). DHR changes the knee angle during foot strike, which means the flexion angle is much greater than in flat level running (Eston et al., 1995). As participants step down the slope, the contracting quadriceps muscle controls the rate of knee flexion against the force of gravity and in the process the muscle undergoes an eccentric contraction with each step (Proske & Morgan, 2001). Furthermore, the highest tension of the anterior and posterior tibial compartments and the hip extensors in the leg extensor muscles are produced whilst the muscles are lengthening when the foot touches the ground (Armstrong et al., 1983). This is more accentuated during DHR (Eston et al., 1995).

There is a vast array of studies that have shown decreased neuromuscular function, ultrastructural damage, DOMS and increased CK activity in the blood following a DHR (see Table 2.1) (Braun and Paulson, 2012, Braun and Dutto, 2003, Byrnes et al., 1985, Chen et al., 2008, Eston et al., 1996). Duration of the DHR protocols utilised varies between 30 minutes and 40 minutes with gradients varying between –9% and –16%. Nevertheless, all studies were able to induce the signs and symptoms associated

with EIMD, suggesting that a DHR protocol is a valid form of muscle damaging exercise.

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Table 2.1 Summary table of studies using downhill running at a method of inducing muscle damage

Authors	Participants	Exercise protocol	Measures & time points	Results
(Braun and Dutto, 2003)	n = 9 (M, well trained distance runners)	30min DHR at -10% gradient with a target intensity of 70% of $\dot{V}O_{2peak}$	Muscle soreness – 0-6 point scale	Muscle soreness was significantly elevated above the baseline measurement at all time points
(Chen et al., 2009)	n = 15 (M, non resistance/endurance trained for 1 year)	30min DHR at -9^0	MVC of knee extensors, perceived muscle soreness, CK measured 3 and 1 days pre- and post- and 5 consecutive days after DHR	DHR resulted in significant development of muscle soreness, ↑ CK that lasts 5 days post DHR
(Chen et al., 2009)	n = 15 (M, non- resistance untrained)	30min downhill run (gradient – 16%) at intensity of pre-determined 70% $\dot{V}O_{2max}$	Muscle soreness, ROM – assessed 5 and 2 days before DHR and at 2 and 5 days after DHR. CK measured 3 and 1 days before DHR, day after and 5 consecutive days after DHR	DHR resulted in significant ↓ in maximal isometric strength, development of muscle soreness, ↑ CK, lasting for 5 d post DHR
(Chen et al., 2008)	n = 50 (M)	-15%	Muscle soreness, CK measure before, after and everyday for 7 days after DHR	All muscle damage markers changed significantly
(Chen et al., 2007c)	n = 10 (M)	30min downhill run (-15%) at 70% $\dot{V}O_{2peak}$ followed by level running at 65, 75 and 85% $\dot{V}O_{2peak}$ (5min each intensity) before, immediately after DHR	VO2 minute ventilation, RER, heart rate, RPE and blood lactate concentration was measured. Stride length, stride frequency, ROM of ankle, knee and hip joints	↓ in maximal isometric strength of the knee extensors, muscle soreness and 1 CK – last for 5 days post DHR

M = male, F = female; MVC = maximal voluntary contraction; PPT = pressure pain threshold; CK = creatine kinase; ROM = range of motion; EF = elbow flexors; EE = elbow extensors; KE = knee extensors; KF = knew flexors; ECC = eccentric exercise; ISO = isometric; VAS = visual analogue scale; VO_2 = volume of oxygen; RPE = rate of perceived exertion; ECC = eccentric contractions; CMJ = countermovement jump; SJ = squat jump

2.3 Exercise-Induced Muscle Damage

It is apparent that eccentric exercise can cause EIMD, predominantly when it is performed in an unaccustomed condition, after an extended break, or with an increase in intensity (Armstrong et al., 1991, Clarkson et al., 1992). EIMD can initiate disruptions in the intracellular muscle structure which can lead to prolonged impairment of muscle function and the development of DOMS (Byrne et al., 2004). The symptoms of EIMD are common and easily characterised, and include a decrease in neuromuscular function (Byrne et al., 2004, Pointon and Duffield, 2012), DOMS (Sellwood et al., 2007), stiffness, (Eston and Peters, 1999), swelling (White and Wells, 2013, Zainuddin et al., 2005), decreased range of motion (Lavender and Nosaka, 2008, Byrnes et al., 2004), elevation of circulating muscle proteins (Nosaka & Clarkson, 1995, Clarkson et al., 1987, Byrnes et al., 1985, Newham et al., 1983), and inflammatory markers (Hunter et al., 2012) (these will be discussed in detail in sections 2.3.1 – 2.3.2). However, the aetiology of these signs and symptoms remain to be elucidated, although several theories have been proposed (Howatson and van Someren, 2008). For the purpose of this literature review the theory of the signs and symptoms of EIMD will be split into two phases of EIMD; the initial phase and the secondary phase.

2.3.1 Phases of EIMD

2.3.1.1 Initial Phase

There are several ways to divide the initial events occurring during EIMD. However, for the purpose of this literature review, the initial phase events that occur as a direct outcome of eccentric exercise will be divided into two sub-divisions; metabolic and mechanical.

2.3.1.2 Metabolic Muscle Damage

Metabolic muscle damage has been proposed to result from ischemia or hypoxia during exercise of a prolonged nature (Armstrong, 1984, Ebbelling and Clarkson, 1989) resulting in an increase in waste accumulation (lactate), changes in ion calcium concentration, and adenosine triphosphate (ATP) deficiency, which ultimately leads to muscle damage (Byrnes & Clarkson, 1986, Howatson & van Someren, 2008). However, Schwane & Armstrong (1983) speculated that the lack of oxygen may only exacerbate the damage from eccentric exercise, with mechanical stress being the main determinant of EIMD. This is supported by Armstrong et al., (1983) who stated that running downhill displayed a high incidence of muscle damage at a lower metabolic cost when compared to uphill running, which requires a higher metabolic cost but results in a much lower incidence of muscle damage. Therefore, metabolic factors, such as a lack of oxygen during exercise, may not be the predominant cause of EIMD. Moreover, this lack of oxygen may be beneficial in increasing the size and number of new mitochondria (mitochondrial biogenesis) (Little et al., 2011). However, the size

and number of mitochondria may be predominantly increased through muscle adaptation to endurance training (Geng et al., 2010), and low-volume high intensity interval training (Little et al., 2011) rather than a single bout of ECC.

2.3.1.3 Mechanical Muscle Damage

Mechanical damage can occur as a direct consequence of the mechanical insult on the muscle fibres (Howatson & van Someren, 2008). This is attributed to the fact that, as the muscle lengthens during ECC, an increased tension is generated and subsequently a higher load is distributed among the same number of fibres, resulting in a higher load per fibre ratio (Clarkson and Hubal, 2002). Furthermore, histological and ultrastructural examination of eccentrically exercised muscle has provided direct evidence of damage to the internal environment of the muscle (Clarkson and Sayers, 1999) such as the muscle fibre contractile, which includes disruption to the Z-line and the sarcomeres (Friden and Lieber, 1992), providing further support that mechanical insult to the fibres is the predominant factor associated with EIMD. The presence of disrupted sarcomeres in the myofibrils results in some myofilaments being stretched to the point where they are unable to overlap within the sarcomere (Talbot & Morgan, 1996, Proske & Allen, 2005, Howatson & van Someren, 2008). This leads to sarcomeres lengthening past optimum length (when a muscle is activated), resulting in the longest sarcomere being stretched more rapidly than others, causing it to become weaker. This causes shearing of myofibrils, exposing membranes to large deformations, resulting in the disruption of calcium homeostasis (Morgan & Proske, 2001), and an efflux of muscle protein, e.g. CK (Sorichter et al., 2001) into the blood stream. In addition to the presence of disrupted sarcomeres in myofibrils, there is also

damage to the excitation-contraction (E-C) coupling system (Proske and Morgan, 2001), which is the process in which contractions in skeletal muscle are translated by the release of Ca^{2+} into the myofibril which initiates the muscle contraction (MacIntosh et al., 2012). This sequence of events begins with the passage of the action potential along the plasmalemma and ends with the release of calcium from the sarcoplasmic reticulum (Warren et al., 2001, Eston et al., 2003). This damage to the E-C coupling system has been demonstrated after eccentric exercise and can explain the disproportionate loss of strength after muscle damaging exercise (Eston et al., 2003). It has been demonstrated that longer duration strength loss associated with eccentric exercise, has been shown to delay the restoration of normal Ca^{2+} levels (Clarkson and Hubal, 2002), which may be related to sarcoplasmic reticulum damage, and can lead to the inability to generate normal force levels (Eston et al., 1995). Furthermore, Desmin, which is a 52-kDA cytoskeletal protein that connects myofibrils at the Z-line, is largely responsible for the lateral transmission of force from contracting muscle (Woolstenhulme et al., 2006). Repeated eccentric contractions may cause failure of the muscle structure, which may results in the reduction in the muscles ability to generate force (Howatson and van Someren 2008). This failure can be translated to a lack of desmin in the skeletal muscle, which results in the loss of anchorage of the myofibrils to the plasma membrane, Moreover; desmin is required for optimal E-C coupling in intact myofibres, and for the long-term maintenance and/or repair of muscle tissue (Paulin and Li, 2004). Research has shown that following muscle damaging exercise there is a decrease in desmin (Friden and Lieber, 2001, Komulainen et al., 1999), which could further explain the decrement in neuromuscular function observed following a bout of eccentric exercise (Paulin and Li, 2004). Subsequently, the mechanical factors associated with the initial phase of

ECC are generally agreed as the primary factors underpinning the development of EIMD.

2.3.2 Secondary Phase

The secondary phase of EIMD develops from the disruption of Ca^{2+} homeostasis due to the shearing of the myofibrils which leads to an elevation of Ca^{2+} in the muscle (Howatson and van Someren, 2008). This consequently leads to further myofibrillar damage in the skeletal muscle (Armstrong et al., 1991, Howatson and van Someren, 2008) by causing alterations to the cytoskeleton, sarcoplasmic reticulum, mitochondria and myofilaments that leads to the degradation of the cell membrane and sarcolemma, cell infiltration and subsequent production of reactive oxygen species, fibre necrosis (Proske & Morgan, 1983, Jones et al., 1986) and ultimately regeneration of the fibres some days later. This further myofibrillar damage leads to degradation of the cell membrane and sarcolemma which then ultimately leads to the repair and regeneration of the muscle tissue (MacIntyre et al., 1996).

Inflammation after muscle injury occurs in order to clear debris from the injured muscles. This is in preparation for regeneration and is characterised by the infiltration of fluid and plasma proteins into the injured tissues (Clarkson & Hubal, 2002). Neutrophils are the first cells to begin accumulating in the muscle, destroying necrotic tissues through phagocytosis (Butterfield et al., 2006). Elevated levels of neutrophils in muscle release proteolytic enzymes and oxygen free radicals that degrade tissue and increase membrane permeability, which allows some cell contents to leak into circulation (Huerta-Alardin et al., 2005). This elevation in circulating neutrophils can

be immediate (Fielding et al., 2000) and documented after various types of eccentric exercise (MacIntyre et al., 2000, MacIntyre et al., 1996). Muscle damage results in the rapid early response by neutrophils, but macrophages develop shortly after (Smith, 2002). This promotes inflammation in order to facilitate the passage of white blood cells from circulation into the muscle tissue (Dinarello, 2000). However, the mechanisms responsible for attracting macrophages remain unknown, and the resulting contributions and interactions of neutrophils and macrophages in tissue repair remains poorly understood (Butterfield et al., 2006).

Overall the development of the disruption of Ca^{2+} homeostasis could result in the signs and symptoms of EIMD due to the increase in inflammation. As mentioned this increase in inflammation consequently leads to further myofibrillar damage in the skeletal muscle contributing to the effects of muscle damaging exercise on the muscles.

2.3.3 The Signs and Symptoms of Exercise-Induced Muscle Damage

EIMD has been defined as a temporary discomfort with 'stiffness' and 'tenderness' (Armstrong, 1984). There are several signs and symptoms associated with EIMD, which can either be measured directly or indirectly. Indirect markers include decrements in neuromuscular function, DOMS, swelling, reduced range of motion (ROM) (Baird et al, 2012), increased limb circumference (Howatson et al., 2012, Chen et al., 2011, Eston et al., 2003), an increase in circulating cytokines into the blood stream (Clarkson and Hubal, 2002) and inflammatory markers (Byrne et al., 2004) which usually transpires 24 - 48 h post muscle damaging exercise (Clarkson et al.,

1992, Cochrane, 2004). Indirect markers are predominantly being used in this study, as they are less invasive compared to direct markers, such as muscle biopsies. Indirect markers of muscle damage can also be inexpensive and are also easily duplicated in the field. However, indirect markers may not demonstrate the true extent of muscle damage after muscle damaging exercise (Byrne and Eston, 2002). Subsequently, EIMD can be characterised when one or more indirect marker is present (Byrne et al., 2004). Furthermore, it has been shown that a single bout of ECC can produce a protective adaptation on subsequent bouts of ECC, characterised by a faster recovery of muscle strength and a smaller development of muscle soreness when ECC is repeated within 1 week to 6 months after the initial bout (Janecki et al., 2011, Nosaka et al., 2001, Chen et al., 2009, Chen, 2003., Chen & Nosaka, 2006, Burt et al., 2013). Additionally, if more than one bout of the same muscle damaging exercise is performed, it has been found that this results in the minimal development of symptoms of muscle damage (McHugh et al., 1999). This is known as the 'repeated bout effect' (McHugh et al., 1999, Burt et al., 2013, Chen, 2003). Therefore, it is important to ensure that when demonstrating EIMD after a single bout of ECC, individuals must be unfamiliar with, and unaccustomed to ECC.

The next section will discuss the indirect markers of EIMD that are measured in this study. These indirect markers include measures of neuromuscular function (squat jump), muscle soreness, range of motion, limb circumference and CK activity in the blood.

2.3.2.1 Indirect Markers of Exercise-Induced Muscle Damage

2.3.2.1.2 Neuromuscular Function

Neuromuscular function can be measured indirectly using maximal voluntary contractions (MVC). MVC is used as a 'gold standard' measure to determine the effect of EIMD on neuromuscular function. Maximum voluntary contractions are considered the most common and reliable method of determining the effects of exercise induced muscle damage on performance (Warren et al., 1999, Byrne et al., 2004). Typically studies have found between 10 and 26% decrease in neuromuscular function (Burt and Twist, 2011, Aldayel et al., 2010), with the general consensus demonstrating a decrease in maximal voluntary contractions (MVC) after a single bout of muscle damaging exercise (see table 2.2). Furthermore, one study demonstrated a 50 to 60% loss of strength, which was not fully restored until 10 or more days later (Clarkson et al., 1992). However, another study reported an immediate 35% reduction in strength, but demonstrated a longer recovery period of 5 - 6 weeks (Howell et al., 1993). Variation in the results of these studies may be due to differences in the participants used, the number of contractions performed during the muscle damaging exercise, the type and duration of the muscle damaging protocols (see table 2.2).

Neuromuscular strength can also be assessed using vertical jump performance (Duthie et al., 2003). Three types of jump can be studied; drop jumps, countermovement jumps and squat jump (Byrne and Eston, 2002). Although, the squat jump is predominantly concentric muscle performance (Byrne and Eston, 2002), reductions in squat jump performance can occur immediately post muscle damaging exercise and can remain

reduced for up to 4 days (Byrne & Eston, 2002). Studies have demonstrated that damaged muscle has a reduced tolerance to impacted force during stretch-shortening cycle (SSC) movement (Clarkson & Newham., 1998). Jakeman et al (2010), found a significant decrease in squat jump, following a muscle damaging protocol, with the greatest decrement in the passive (CON) group. It is suggested that the SSC is impaired following a downhill run (Eston et al., 1995), which may contribute to the decrease in squat jump performance. This decrease in squat jump performance could further relate to decreases in performance in a sporting scenario or in a game situation that requires the action of jumping e.g. basketball.

2.3.2.1.3 Muscle Soreness

DOMS is the development of muscle soreness, which usually peaks between 24 and 48 h post muscle damaging exercise (Rowlands et al., 2012, Smith et al., 1994). Although the theories of DOMS remain to be elucidated, DOMS is classified as a type I muscle strain injury that presents with tenderness, or stiffness during palpitation or movement (Cheung et al., 2003). Muscle soreness is probably the most recognised and commonly used indicator of muscle damage among untrained and trained populations (Jones et al., 1986, Warren & Armstrong, 1999). The sensations experienced can range from slight muscle stiffness, which rapidly disappears during daily routine activities, to severe debilitating pain which restricts movement (Cheung et al., 2003). Tenderness is typically concentrated in the distal portion of the muscle and becomes progressively diffused by 24 – 48 h post exercise (Armstrong, 1984, Armstrong & Warren, 1993, Cheung et al., 2003). This localisation of pain can be attributed to a high concentration of muscle pain receptors in the connective tissue (Cheung et al., 2003).

Conversely, research has suggested that DOMS may be a result of the inflammatory response following muscle damaging exercise (Smith, 1991). Prostaglandins, histamines and bradykinins have been implemented in producing the sensation of soreness as they are released when muscle tissue is damaged. Subsequently, they activate type III and type IV nerve afferents that carry messages of pain from the muscle to the central nervous system (O'Connor & Cook, 1999, Clarkson & Hubal, 2002). This can further be increased by an increase in intramuscular pressure brought on by movement (Cheung et al., 2003, Armstrong, 1984, Smith, 1991). However, there is no direct evidence that these pain receptors produce muscle damage (Clarkson & Hubal, 2002). It is speculated that the muscle soreness originates from the lowering of the threshold due to the sensitisation of muscle pain receptors (Clarkson & Hubal, 2002). One theory of DOMS suggests that lactic acid, which continues to be produced following exercise cessation, in addition to the accumulation of toxic metabolic waste products, causes an increase in perception of pain (Armstrong, 1984). However, lactic acid levels return to pre-exercise baseline within an hour following DHR (Schwane et al., 1983). Therefore, lactic acid cannot be attributed to the delayed pain that is experience 24 - 48 h post muscle damaging exercise. Another theory proposes that the mechanical disruption of Z-lines and damage to the sarcolemma increases the diffusion of CK into the interstitial fluid, which could lead to induced soreness (Cleak and Eston, 1992a). However, literature demonstrates a clear discrepancy between peak CK activity and peak muscle soreness (Newham et al., 1986, Newham et al., 1983).

Table 2.2 shows a multitude of studies that demonstrate an increase in muscle soreness post muscle damaging exercise (Clarkson et al., 1992, Davies et al., 2009, Davies et

al., 2008, Davies et al., 2011a, Doncaster and Twist, 2012, Howell et al., 1999), with muscle soreness peaking between 1 and 3 d post muscle damaging exercise (Davies et al., 2011a, Clarkson et al., 1992) and increases in muscle soreness being reported as early as 30 min post damaging exercise (Davies et al., 2009). There are several methods of measuring muscle soreness and VAS has been shown to be a valid, reliable, and commonly used measurement tool (Cleather et al., 2007). Demonstrated in Table 2.2 it is apparent that studies employing squats (Burt et al., 2012, Davies et al., 2011, Davies et al., 2009, Davies et al., 2008), plyometric exercise (Burt and Twist, 2011, Jakeman et al., 2010), drop jumps (Goodall and Howatson, 2008), and knee extensions (Aminian-Far et al., 2011) as a muscle damaging protocol produced peak muscle soreness at 48 h compared to high intensity running (Bailey et al., 2011) and downhill running (Chen et al., 2007, Nottle and Nosaka, 2007), which results in peak muscle soreness at 24 h post muscle damaging exercise. It is unsure as to why this occurs, but as previously mentioned, it may be due to the fact that downhill running requires a lower metabolic cost which results in a lower extent of muscle damage (Armstrong et al., 1983), which therefore suppresses the secondary increase in inflammation caused by further disruption of Ca^{2+} homeostasis (Howatson and van Someren, 2008), which leads to further myofibrillar damage in the skeletal muscle (Armstrong et al., 1991, Howatson and van Someren, 2008). However, variation in findings may also be due to the subjective nature of muscle soreness, as perceived pain can be affected by the emotions of the participant on the day of testing (Nosaka et al., 2002). Not only the subjective views on pain may cause variations it could be argued that subjective responses to soreness may also cause variations to findings (Nosaka et al., 2002)

A variety of pain scales have evolved to quantify DOMS, with different levels of reliability depending upon the method used to assess the perception of pain (Cleak & Eston, 1992). For this study a pain scale of 1 – 10 was used (Cook et al., 1997) as this is a reliable method of determining an individual's perception of pain. Therefore, it is essential to develop a method of quantifying the development and severity of muscle 'tenderness' (Newham et al., 1983) over several different areas of damaged muscles (Newham et al., 1987, Newham et al., 1983, Jones et al., 1987, Newham et al., 1988) to ensure an accurate and global overview of DOMS is obtained. Pressure pain threshold can be measured using a pressure pain algometer, as demonstrated in Rowlands et al (2012) study, which found that there was an increase in muscle soreness post exercise (run with a mean distance of 119.6 km), particularly in the quadriceps (Rowlands et al., 2012). Another study demonstrated a greater decrease in pressure pain threshold scores in the control group (74%) when compared to the non-control group (38%) at 24 h, indicating that the muscle damaging protocol (6 x 10 maximal isokinetic eccentric knee extensions) was successful in inducing muscle damage (Aminian-Far et al., 2011). Moreover, these signs and symptoms develop with increasing intensity which has been observed as early as 1 h after exercise (Clarkson and Tremblay, 1988) with symptoms peaking 24 – 72 h after the muscle damage protocol.

2.3.2.1.4 Range of Motion and Limb Circumference

Decreases in ROM correspond with increases in perceived soreness (Isabell et al., 1992). It is speculated that this decrease in range of motion could be related to the loss in muscle strength which is compatible with the over stretch sarcomere theory (Clarkson et al., 1992). The shortening of the connective tissues and the muscle fibres

or changes in the tendons at the attachments may also contribute to the shortening of the muscles (Clarkson et al., 1992, Jones et al., 1987, Newham, 1988). By measuring limb circumference, it can be found that swelling gradually develops in the days after ECC exercise, reaching peak values at 5 days post muscle damaging exercise (Clarkson et al., 1992).

2.3.2.1.5 Creatine Kinase

CK is a compact enzyme that is found in both the intracellular fluid and the mitochondria of tissues where energy demands are high (Baird et al., 2012). CK buffers cellular ATP and adenine diphosphate concentrations by catalysing the reversible exchange of high-energy phosphate bonds between phosphocreatine and ADP produced during contraction (Brancaccio et al., 2007). This increased permeability allows for a greater influx of CK (Pizza et al., 1995) to enter the blood and remain elevated, which can be seen in the blood for several days post exercise (Howatson and van Someren, 2008, Chen, 2006, Baird, 2012). Although the general consensus is that CK is used as a marker of muscle damage, the presence of CK in the blood may not accurately indicate the extent of muscle damage, as its presence may not actually reflect the release rate or the rate of removal from the blood (Wilcock et al., 2006a, Zainuddin et al., 2005). Furthermore, several studies have shown that CK does not reflect the magnitude of damage (Nosaka et al., 2002, Clarkson & Hubal, 2002, Clarkson et al., 1992, Evans & Cannon, 1991). Nevertheless, a plethora of studies use CK as a marker of EIMD (Chen & Nosaka, 2006, Nosaka & Clarkson, 1994, Newham et al., 1986, Nosaka & Clarkson, 1996). As demonstrated in Table 2.2, research has shown large increases in CK concentrations in the blood after eccentric

exercise (Newham et al., 1983, Nosaka et al, 1991, Nosaka & Clarkson, 1992, Nosaka & Priscilla, 1994). Additionally, Chen (2006) demonstrated that CK can range from 96 to 34,500 IU/L, suggesting large intra-individual differences in CK values. However, to date there is still no clear explanation for CK variability after a single bout of eccentric exercise. The magnitude of change in CK may be dependent on sex, ethnicity, training status and muscle damaging protocol employed (Brancaccio et al., 2007). For example, overall, CK activity are generally lower in females, which may be due to oestrogen maintaining post-exercise membrane stability, limiting CK release from damaged muscle (Tiidus, 2000, Brancaccio et al., 2007). Additionally, CK concentrations are usually higher in black men who demonstrate high body weight and leaner body mass (Schuttle et al., 1984); however, this may not correlate with increased levels of CK post muscle damaging exercise (Brancaccio et al., 2007). The underlying mechanisms of increased CK in the blood and its association with the extent of muscle damage are equivocal (Nosaka & Clarkson, 1994, Manfredi et al., 1991), and subsequently caution should be taken when using this measurement tool as a marker of EIMD and recovery. Therefore, the increase in CK may not contribute to the increase in muscle soreness post muscle damaging exercise (Clarkson et al., 1992, Clarkson & Hubal, 2002). This is supported by the notion that CK is not a direct indicator of the extent of muscle damage (Evans & Cannon, 1991). Although CK may be a questionable method of determining muscle damage or may not accurately indicate the extent of muscle damage, CK is used in the study due to the fact that the majority of studies that look at muscle damage and recovery use CK as an indicator, therefore making it easy to compare this study with other studies.

2.3.4 Summary

It is apparent that unaccustomed eccentric exercise induces signs and symptoms of EIMD e.g. DOMS, which can negatively affect athletic performance due to the increase in perceived pain or discomfort (Armstrong, 1984, Cleak and Eston, 1992a, Byrnes et al., 1985). Complete recovery and restoration of muscle function following ECC may take a week or longer (Clarkson & Tremblay, 1988, Friden et al., 1983, Newton et al., 1987). For example, Friden et al., (1983) found evidence of ultrastructural damage in biopsies taken immediately and 3 days after subjects performed ECC cycle ergometer exercise. Furthermore, 6 days post exercise, disturbances were still evident in 12% of the observed fibres. Furthermore, Clarkson & Tremblay, (1988) monitored DOMS, ROM, and CK following ECC and found no measure had returned to baseline within 5 days post exercise. This evidence suggests that recovery from ECC is a relatively slow process (Ebbelling & Clarkson, 1990). Therefore, it is vital to determine an adequate treatment strategy, such as water immersion to help alleviate the signs and symptoms associated with EIMD in order to restore maximal function of muscles as rapidly as possible (Cheung et al., 2003). Furthermore, it may also be important to alleviate DOMS as this could be associated with less than optimal performance. This is especially important during multiple day tournaments when athletes need to restore the maximal function of muscle as rapidly as possible in order to optimise their performance.

Table 2.2 Summary table of studies that use different methods of eccentric exercise to induce muscle damage

Authors	Participants	Exercise Protocol	Measures & time points	Results
(Aldayel et al., 2010)	n = 9 (M, non resistant trained for at least 6 months)	2 bouts of knee extensors of one leg stimulated by biphasic rectangular pulses at knee joint angle of 100° 2 weeks apart	MVC torque of the knee extensors at 100°, VAS, PPT and CK before and 1, 24, 48, 72, 96 h	↓ in MVC by 26% after and 1 h after both bouts. Recovery significantly faster after second bout. PPT was significantly smaller after the second bout.
(Aminian-Far et al., 2011)	n = 32 (10 = M, 22 = F, untrained)	2 session separated by 2 to 3 days – 2 trials of perceived maximal isometric and eccentric actions	MVC, isokinetic knee extensor strength, limb circumference, PPT, CK and muscle soreness. Before, immediately after exercise on days 1,2,3,4, 7 and 14 post exercise	No difference between groups. ↓ Maximal ISO torque, ↓ torque in control group, ↓ in control group in PPT. No difference in CK between groups
(Bailey et al., 2011)	n = 38 (M, non-smokers, habitually active, unfamiliar with specific exercise protocol	~90min intermittent shuttle-running	Isometric MVC knee extensors & flexors (non-dominant leg) pre, immediately, 24, 48, 96 and 168 h post exercise	↓ in MVC of extensors and flexors at all time points in both groups
(Burt et al., 2012)	n = 9 (M)	Squats (100 squats at 80% body mass on Smith-machine, 2 min rest)	Isokinetic MVC knee extensors ($60^{°s\text{-}1}$) pre, 24 and 48 h post exercise	↓ in MVC at all time points, peak ↓ 48 h post exercise

M = male, F = female; h = hour; MVC = maximal voluntary contraction; PPT = pressure pain threshold; CK = creatine kinase; ROM = range of motion; EF = elbow flexors; EE = elbow extensors; KE = knee extensors; KF = knew flexors; ECC = eccentric exercise; ISO = isometric; VAS = visual analogue scale; SJ; squat jump; BW = body weight

Table 2.2 continued Summary table of studies using eccentric exercise as a form of muscle damaging exercise

Authors	Participants	Exercise Protocol	Measures & time points	Results
(Burt and Twist, 2011)	n = 17 (M = 15, F = 2)	Ramp protocol to exhaustion Plyometric jumps (10 x 10)	Isokinetic knee extension ($60°·s^{-1}$) pre, 48 h post exercise	↓ in MVC ~10% 48 h post exercise
(Chen et al., 2011b)	n = 17 (M, sedentary)	5 x 6 maximal isokinetic eccentric contractions of elbow flexors and extensors and knee flexors and extensors	Isometric and concentric isokinetic strength, optimum angle, limb circumference, ROM, CK and muscle soreness pre, immediately, 1,2,3,4 and 5 days post exercise	↓ in isokinetic MVC immediately post exercise in EF (~33%), EE (~30%) KF (~17%) and KE (~5%). ROM ↓ after ECC exercise
(Chen et al., 2011a)	n = 30 (M, non resistance, aerobic or flexibility training)	6 x 10 maximal lengthening contractions of left knee flexors	ROM, VAS – before and 3 day after last flexibility training session, immediately before and after and 24h interval for 5 days. CK – measured before and 1-5 days after exercise	↑ (25°) ROM after 8 wk flexibility training
(Chen et al., 2012)	n = 65 (M, non resistance trained	30 ECC of the elbow flexors using dumbbell equivalent to 100%, 10% and 20% of MVC strength	ROM, limb circumference, CK & muscle soreness before and for 5 d after the first and second bout	All variables changed significantly after maximal eccentric exercise

See table 2.2 for list of abbreviations

Table 2.2 continued Summary table of studies using eccentric exercise as a form of muscle damaging exercise

Authors	Participants	Exercise Protocol	Measures & time points	Results
(Clarkson et al., 1992)	n = 109	Maximal ECC action of forearm flexor muscles (2 x 35) every 15s, 5min rest between the 2 sets 50-60% loss in strength – 10 days to reach baseline levels	Muscle soreness, ROM, CK – pre and 1-10 d post exercise	Muscle soreness peaked 2-3 d post exercise, swelling peak occurs 5 d post exercise. CK ↑at 2 d post exercise 50-60% loss in strength – 10 days to reach baseline levels
(Davies et al., 2011b)	n = 10 (M, physically active)	Eccentric exercise 100 squats (Smith-squats at 70% of BW)	Isokinetic MVC knee flexors (30̊ s-1) pre, 30min, 24 and 48h post exercise	↓ in MVC ~20%, ~17% and ~15% 30 min, 24 and 48 h post exercise respectively
(Davies et al., 2009)	n = 10 (M, physically active)	100 squats with a load corresponding to 70% of body mass (10x10)	Muscle soreness before, 30min and 48h post ECC exercise. CK measures before, 30min, 24 and 48h post	Soreness reported 30min post ECC exercise, ↑ in CK activity with highest value observed at 24h
(Davies et al., 2008)	n = 9 (M)	100 smith squats (10x10) load corresponding to 70% of body weight	CK, perceived muscle soreness (VAS) immediately before, 24 and 48h post exercise.	Significant changes in all markers of muscle damage. Highest value of muscle soreness was 48h. ↑ CK, highest value observed at 24h
(Davies et al., 2011a)	n = 10 (M, physically active)	100 smith squats, (10x10) load corresponding to 70% BW	CK, perceived muscle soreness (VAS) immediately before, 24 and 48h after exercise	Muscle soreness increased 24h after exercise, highest value reported at 48h. ↑ CK, highest value observed at 24h

See table 2.2 for list of abbreviations

Table 2.2 continued Summary table of studies using eccentric exercise as a form of muscle damaging exercise

Authors	Participants	Exercise Protocol	Measures & time points	Results
(Highton et al., 2009)	n = 12 (M, healthy, recreationally active)	100 plyometric jumps (10x10) with 1min rest	Perceived muscle soreness recorded at baseline, 24, 48 and 168h following ECC exercise	No significant differences observed between treatments and control group for baseline values. Muscle soreness group significantly higher that baseline at 24 and 48h
(Jakeman et al., 2010)	n = 17 (F)	10 x 10 Plyometric drop jumps, with 1 min rest	Muscle soreness, CK, SJ assess prior to and 1, 24, 48, 72 and 96 h post ECC exercise	Significant on all indices of muscle damage. ↓ in MVC at all time points, greater ↓ in passive group, peak ↓ 48 h post exercise ↓ in SJ at all time points in both groups, greater ↓ in passive group ↓ in SJ 1, 24 and 48 h post exercise in both groups, greater ↓ in passive group 48 h post exercise, no change at any other time point in either group
(Newham et al., 1983)	n = 4 (3 M & 1 F)	Step test for 20min – relative height for each subject	Muscle biopsies	Muscle strength returns to pre-exercise levels within 24h

See table 2.2 for list of abbreviations

2.4 Section 2: Introduction to Water Immersion used as a Recovery Method

Water Immersion has increasingly been utilised as a method of recovery after exercise, post-game or post-training due to the non-exercising element (Wilcock et al., 2006), even though much of its use is based on anecdotal information (Calder, 2003). This section of the literature review will firstly focus on the physiological mechanisms of the temperature of the water, albeit cold water immersion, contrast water therapy, and hot water immersion. Secondly, there will be a focus on the effect of hydrostatic pressure in thermoneutral water as a separate intervention.

2.4.1 Physiological Mechanisms Underpinning the use of Water Immersion to Alleviate the Signs and Symptoms of DOMS

Water immersion has gained popularity as a means of improving recovery from exercise (Wilcock et al., 2006a) to reduce the signs and symptoms of EIMD (Poppendieck et al., 2013) and allow greater subsequent training loads (Versey et al., 2013). Although there is a scarcity of literature, research has proposed that water immersion may assist in attenuating the signs and symptoms of EIMD through a combination of mechanisms of hydrostatic pressure and temperature (Wilcock et al., 2006a). However, it is a question as to whether these two mechanisms can alleviate the signs and symptoms of EIMD individually or in conjunction with each other. It is also a question of whether HWI and CWT is more ecologically valid, thus allowing individuals to tolerate the water immersion protocols more so than the CWI and still obtain benefit results. CWT and HWI may be more ecologically valid than CWI due to the extreme temperature and long duration of the CWI, some athletes or individuals

may not be able to tolerate extreme cold temperatures long enough for the cold water to have an effect (decrease in muscle temperature, decrease in blood flow).

The next section will discuss the mechanisms of water temperature and hydrostatic pressure separately in order to distinguish the effects of water temperature on the body and the effect of hydrostatic pressure on the body during immersion. Please note that although thermoneutral water temperature was not investigated within this study, in the hydrostatic pressure section, water temperature will be thermoneutral to minimise the effects of dramatic decreases or increases in temperature.

2.4.1.1 Mechanisms of Different Water Temperatures

2.4.1.1.2 Mechanisms of CWI

The exact mechanisms of CWI are speculated to be related to temperature induced changes within the muscle (Leeder et al., 2012, Bleakley and Davison, 2010), and subsequently reduced post-exercise inflammation (Yanagisawa et al., 2003). Exposure to critically cold temperatures can results in decrease heart rate leading to decrease in cardiac output and an increase in peripheral resistance caused by blood being redirected from the peripheries to the core (Sramek et al., 2000, Bonde-Petersen et al., 1992). Cold water immersion is thought the reduce the permeability of cellular, lymphatic and capillary vessels through vasoconstriction (Ascensao et al., 2011) which is thought to reduce inflammation by reducing the fluid diffusion into the interstitial space (Wilcock et al., 2006, Leeder et al., 2012). This reduction in inflammation may enhance recovery and help in alleviating the signs and symptoms

of EIMD (Swenson et al., 1996), and subsequently improve performance. Cooling the muscle through CWI may reduce the release of calcium from the sarcoplasmic reticulum (Davies et al., 1982), which may be important, as a reduction in calcium homeostasis induces the secondary phase of muscle damage (Armstrong et al., 1991) Subsequently, CWI may delay or prevent the second phase from occurring, which ultimately can improve recovery or result in less DOMS. Furthermore, CWI has been demonstrated to decrease the concentration of CK following EIMD (Ascensao et al., 2011, Eston and Peters, 1999, Ingram et al., 2009, Vaile et al., 2008b) (see Table 2.6). The mechanism attributed to this decrease in CK following CWI is a decrease in cellular, lymphatic and capillary permeability caused by vasoconstriction induced by a decrease in temperature (Wilcock et al., 2006a). If CWI is not implemented there is an increase in CK in the blood due to this increase permeability of the membrane (Huerta-Alardin et al., 2005, Pizza et al., 1995, Howatson and van Someren, 2008), which is apparent in some studies in table 2.6 where there was a decrease in CK concentration in CWI groups compared to a control or thermoneutral group. Additionally, due to vasoconstriction and a decrease in nerve conductions in the muscle (Abramson et al., 1966) cold water immersion decreases swelling and pain (Smith, 1991), which are both important signs and symptoms of EIMD that can negatively affect subsequent performance. Another aspect of CWI is the temperature of the water and the duration of the immersion. As demonstrated in Table 2.4 and Table 2.6, several studies have used temperatures of $\leq 15^0$C in performance based studies (Bailey et al., 2007, Eston and Peters et al., 1999a, Halson et al., 2008, Montgomery et al., 2008) and durations ranging from 5 min (Higgins et al., 2011) to 24 min (Kuligowski et al., 1998) to intermittent immersions protocols (King and Duffield, 2009, Robey et al., 2009, Rowsell et al., 2011). However, although there is

an increase in literature on the effects of CWI recovery, findings on potential benefits remain equivocal (Ingram et al., 2009, Rowsell et al., 2009, Pointon and Duffield, 2012).

2.4.1.1.3 Mechanisms of HWI

In contrast to CWI which decreases the permeability of cellular, lymphatic and capillary vessels reducing inflammation, HWI does the opposite and increases the permeability of these vessels. This increase in permeability increases the metabolism, nutrient delivery and waste removal from the muscle tissues and cells, ultimately leading to increased healing (Cote et al., 1988, Wilcock et al., 200). Immersion in hot water will increase blood flow (Hing et al., 2008), and tissue temperature (Enwemeka et al., 2002), due to the vasodilatory mechanisms. Superficial heat related recovery treatments have shown to increase tissue temperature (Kubo et al., 2005), increase local blood flow (Cochrane, 2004, Stanton et al., 2003), increase muscle elasticity (Coffey et al., 2004), cause local vasodilation (Vaile et al., 2007), reduce muscle spasm (Cochrane, 2004, Wilcock et al., 2006a), which all in turn will increase the healing process after muscle damaging exercise (Wilcock et al., 2006a). Furthermore, this will augment the signs and symptoms of EIMD by increasing joint extensibility, increasing neural transmission proprioception, and improving reaction time (Wilcock et al., 2006a). Therefore, these aforementioned changes should lead to an improvement in performance. However, during HWI, unlike CWI there is no vasoconstriction, which may be required in order to alleviate the signs and symptoms of EIMD through vasodilation, which increases the amount of blood flow and reduces inflammation. For this reason, it is questionable as to whether hot water immersions

alone will have a beneficial effect on alleviating the signs and symptoms of EIMD without the vasoconstriction action of CWI to decrease inflammation. Therefore, it is probable that with the combination of both HWI and CWI, there will be a propitious outcome, when compared to the single temperature treatments.

2.4.1.1.4 Mechanisms of CWT

It is proposed that through CWI there is a decrease in inflammation and through HWI there is an increase in blood flow and waste removal. Therefore, optimal vasoconstriction and vasodilation may be achieved by CWT, which minimises swelling and soft tissue damage through vasoconstriction of CWI, impairing the inflammatory response (Myrer et al., 1994). CWT is widely utilised (Cochrane, 2004) and considered to enhance recovery through; stimulating area-specific blood flow (Hing et al., 2008), increasing blood lactate removal (Hamlin, 2007), reducing inflammation and oedema (Enwemeka et al., 2002), stimulating circulation (Myrer et al., 1994), relieving stiffness and pain (Cochrane, 2004), increasing range of motion (Cote et al., 1988) and reducing DOMS (Wilcock et al., 2006a, Hing et al., 2008, Cote et al., 1988). It is also believed that CWT replicates the mechanisms of active recovery without the same energy demands (Wilcock et al., 2006a, Cochrane, 2004), which is essential during multiple day tournaments or competition in order to enhance the recovery process without risking the chance of inducing more muscle damage through active recovery (such as jogging). Furthermore, the immersion in hot water has been shown to increase tissue temperature, metabolite production and muscle elasticity, to stimulate local blood flow and to reduce muscle spasm (Cochrane, 2004, Hing et al., 2008).

In contrast to the effects of water temperature, it has been proposed that hydrostatic pressure can also produce beneficial physiological changes within the body (Wilcock et al., 2006). The effects of hydrostatic pressure on the body during water immersion will be demonstrated in the next section.

2.4.1.2 Mechanisms of Hydrostatic Pressure

It has also been speculated that hydrostatic pressure alone may contribute to the beneficial effects of water immersion, without the element of drastic increases or decreases in water temperature. The use of thermoneutral water may avoid the intolerance to extreme cold water or the detrimental effects of hot water immersion (Wilcock et al., 2006)

Hydrostatic pressure is a force exerted on the body when immersed in water. This pressure may limit swelling, pain, and a loss of force production (Smith, 1991) following exercise due to the reduced permeability of cellular, lymphatic and capillary vessels as a result of vasoconstriction, which reduces fluid diffusion into the interstitial space (Eston and Peters, 1999), potentially reducing further muscle damage (Wilcock et al., 2006a). The displacement of fluids from the extremities towards the central cavity, may increase the translocation of substrates from the muscles, increase cardiac output (increased blood flow throughout the body), and increase the ability of the body to transport substrates, diffusions of metabolic waste products from muscle to blood (Wilcock et al., 2006a, Sramek et al., 2000). Furthermore, there may be an improvement in the reabsorption of interstitial fluids, which in turn will reduce

swelling (which we already know is a contributing factor of DOMS) (Friden & Lieber, 2001, Wilcock et al., 2006). Subsequently, the reduction in swelling may enhance nutrient delivery and increase the rate of recovery from exercise that has induced muscle damage (Wilcock et al., 2006). Wilcock et al., (2006) stated that the main effect of water immersion (after eliminating the effects of water temperature) comes from the effects of hydrostatic pressure, therefore, it is possible that the combined effects of hydrostatic pressure and water temperature may produce a greater beneficial effect on alleviating the signs and symptoms of EIMD and improving recovery. However, the effect of hydrostatic pressure used to reduce delayed onset muscle soreness and to attenuate the detrimental effects of exercise on subsequent performance remains equivocal (Ingram et al., 2009). There are also links that CWT may induce changes in intra-muscular hydrostatic pressure by alternating vasoconstriction and vasodilation, which may alter blood flow (Ingram et al., 2009). Furthermore, the recovery benefits of CWI reported by Ingram et al. (2009) study are most likely due to water temperature rather than hydrostatic pressure.

2.4.2 Water Immersion as a Recovery Intervention from EIMD

Table 2.4 shows a summary of literature that demonstrates the effects of different water immersion strategies (CWI, hydrotherapy, whirlpool therapy, CWT and HWI) on the signs and symptoms of EIMD after eccentric exercise. Numerous studies (Montgomery et al., 2008, Rowsell et al., 2009, Rowsell et al., 2011, Bailey et al., 2007, Eston and Peters, 1999a, Kuligowski et al., 1998, Vaile et al., 2008b, Yanagisawa et al., 2003) show that cold water immersion has beneficial effects on the signs and symptoms of EIMD. Conversely, several studies found no beneficial effects

of CWI on the recovery from EIMD (Jakeman et al., 2009, Halson et al., 2008, Sellwood et al., 2007, King and Duffield, 2009) (See table 2.5), and one study only found a significant reduction in muscle soreness 24 h post exercise (Ingram et al., 2009). Variation in the findings of these studies could be due to different muscle damaging protocols, interventions implemented, water temperature, immersion duration, and performance measures, which makes the comparison of studies problematic. Furthermore, establishing the 'optimum' water immersion protocol required to attenuate the performance decrements associated with the signs and symptoms of EIMD are extremely difficult.

It is also apparent from Table 2.4 that there is a general decrease in muscle soreness following both CWI and CWT compared to no water immersion (Bailey et al. 2007, Ingram et al., 2009). Additionally, CWI facilitates a rapid return to baseline levels post muscle damaging exercise (Ingram et al., 2009) compared to no water immersion. For example, Vaile et al., (2008b) found that decrements in squat jump peaked at 48 h post CWT compared to 72 h following passive recovery. This means that with the addition of CWT, individuals are then able to return to optimum performance levels quicker than if no recovery intervention was implemented. Furthermore, CWI was also more effective at decreasing the perception of muscle soreness and enhancing the sensation of recovery (Halson et al., 2008). According to table 2.4, the exercise protocols (squats, plyometric exercise, box jumps, knee extensions, game simulations maximal ECC contractions and intermittent sprint tests) were effective in producing muscle damage as there was an increase in muscle tenderness and a decrease in isometric force, sprint performance and maximal voluntary contractions. Therefore, it can be suggested that CWT and CWI may enhance both the perceptual and physiological

symptoms of EIMD, which could be beneficial for recovery during multi-day competitions. Studies that demonstrate either or both interventions of CWT and CWI found reductions in muscle soreness ratings as well as increase in muscle tenderness post muscle damaging protocol.

In contrast to the beneficial effects of CWI and CWT, both HWI and CWT have been shown to increase oedema when compared with CWI (Cote et al. 1988). However, these authors were investigating the effects of water immersion for minimising ankle sprain injury rather than muscle damage caused by exercise. Subsequently, CWI which utilises both vasodilatory and vasoconstriction mechanisms could be beneficial for reducing the signs and symptoms of EIMD. Unfortunately, only one study by Vaile et al (2008b) focused on HWI (Table 2.6). Vaile and colleagues found these findings may not translate to EIMD, and therefore, further work should investigate whether HWI and CWT can reduce the signs and symptoms associated with muscle damaging exercise.

Several studies have shown an improvement in the signs and symptoms of EIMD following CWT (Kuligowski et al., 1998, Vaile et al., 2008b, Higgins et al., 2011). However, the majority of the studies have shown no beneficial effect of CWT, when compared to a control condition (Jakeman et al., 2009, King & Duffield, 2009, Sellwood et al., 2007). All studies in Table 2.6 employing CWT begin the protocol with CWI, which encourages vasoconstriction (Cochrane, 2004) which promotes decreases in inflammation. There is a plethora of research that has demonstrates the alternation between cold water and hot water, with each immersion lasting between 30-120 seconds, repeated 2-5 times (Coffey et al., 2004, Cote et al., 1988, Fiscus et

al., 2005, Gill, 2006, Hamlin, 2007, Higgins et al., 2012, Higgins and Kaminski, 1998, Kuligowski et al., 1998, Vaile et al., 2007, Vaile et al., 2008b). However, one study demonstrated intramuscular temperature did not fluctuate with repeated 1 min immersion into cold water following 4 min of water immersion (Wilcock et al., 2006a). If deep tissue temperature does not change with alternating immersion, any vasopumping would then be likely to occur at a subcutaneous level only, restricting recovery and intramuscular metabolic removal as these changes would need to occur at a deeper tissue level (Wilcock et al., 2006a). The means that CWI, HWI and CWT, may not have beneficial effect of exercise induced muscle damage due to the limited exposure times. The limited amount of time exposed to cold, hot or contrast may not be beneficial enough to allow a change in temperature in the deep tissues of the damaged muscle, which could affect the rate of recovery after muscle damaging exercise

Table 2.5 shows several studies that challenge the wide use of water immersion as a means of reducing the effects of EIMD, as both CWI and CWT show no beneficial effect on alleviating the signs and symptoms of exercise induced muscle damage. Variation in these findings compared to Table 2.4 could be due to the fact that these studies show that exercise was not eccentrically based, and immersion times were short, also the studies used highly trained participants, which may result in participants not experiencing the same level of muscle damage as they may be accustomed to that specific exercise (Jakeman et al., 2009, King and Duffield, 2009). Both studies utilised short immersion protocols (10 min and 5 min cold water immersion and hot shower). The use of the hot showers eliminated the hydrostatic pressure effect, which could also help explain why there was no significant difference in the signs and

symptoms of EIMD following water immersion. Sellwood et al (2007) study used untrained participants (n = 40) and still failed to observe any beneficial effect of water immersion on the signs and symptoms of EIMD. However, this may be due to the minimal exposure times (3 x 1 min, with 1 min rest in between).

Differences in the findings of these aforementioned studies could be as a result of several factors including variation in cooling methods (e.g. CWI, CWT, hydrotherapy, cryotherapy), cooling durations (e.g.1 min – 40 min), types of performed exercise (e.g. countermovement jump, DHR, drop jumps, and simulated games), and different performance measures (e.g. sprints, squat jump, MVC) as well as different sample sizes (n = 8 – 56) and population demographics (e.g. trained vs. untrained) (Howatson & Van Someren, 2008). For example as shown in Table 2.6 there is a wide variety of different CWT treatment protocol, with different ratios, duration and temperatures (Coffey et al., 2004, Hamlin, 2007, Kuligowski et al., 1998, Myrer et al., 1994, Myrer et al., 1997, Robey et al., 2009, Vaile et al., 2008a, Vaile et al., 2008b). Cold water temperature ranges between 10^0C - 15^0C and hot water temperature between 38^0C - 40^0C. Furthermore, the duration of immersion ranges from 1 - 10 min immersed and 1 – 10 min out of the water. Therefore, the comparison of studies is problematic.

Table 2.3 Summary table of the effects of different water immersion strategies on the signs and symptoms of exercise induced muscle damage

Authors	Participants	Exercise Protocol	Treatment protocol and measurements	Results
(Bailey et al., 2007)	n = 20 (M)	90min intermittent shuttle run	10 min CWI (10^0C), CON RPE, changes in muscular function, CK before exercise, during treatment,	↓muscle soreness at 1, 24, and 48 h post ↓MVC after CWI at 24h CK peaked at 1 and 24 h – CWI had no affect on CK
(Eston and Peters, 1999a)	n = 15 (F)	Elbow flexors – isokinetic dynamometer	CON, CWI (15^0C) for 15min post exercise, ever 12h for 7 sessions. Muscle tenderness, CK, ROM, isometric strength, LC – before, for 3 days post	↑ in muscle tenderness, CK and LC ↓ isometric force, ROM Sig interactions of group x time ROM (↑) and CK (↓) for CWI 24, 48h
(Halson et al., 2008)	n = 11 (M, endurance trained cyclist)	40min time trials	CWI (11.5^0C, 3 x 60s), CON, CK, T_{skin} measured throughout	Reported enhanced sensations of recovery following CWI – no sig diff
(Ingram et al., 2009)	n = 11 (M, team sport athletes)	3 day testing trials, separated by 2 weeks – 80 min of simulated team sports followed by 20m shuttle run test to exhaustion	CWT, CWI 10m by 20m sprint, ISO recorded day 1 After, 24h post-exercise recovery for 15min 48h performance measures were repeated	↓reduction in muscle soreness ratings and ISO leg extension at 48h in CWI, also facilitated rapid return to baseline repeated spring performance CWT only ↓in muscle soreness 24h post exercise

M = male, F = female; CWI = cold water immersion; RPE = rate of perceived exertion; CK = creatine kinase; CON = control; MVC = maximal voluntary contraction; ROM = range of motion; CWT = contrast water therapy; PAS = passive recovery; LC = limb circumference; HWI = hot water immersion; ISO = isometric force contractions

Table 2.3 continued Summary table of studies demonstrating the effects of different water immersion strategies following exercise induced muscle damage

Authors	Participants	Exercise Protocol	Treatment protocol and measurements	Results
(Kuligowski et al., 1998)	n = 56 (M = 28, F = 28)	Eccentric contractions of elbow flexors , 24min warm whirlpool (38.90°C), cold whirlpool (12.8°C), CWT (38.9°C and 12.8°C) and no treatment	0h- pre, 24, 48 and 72h- post and 96h post, ROM, perceived soreness, MVC	Cold whirlpool & CWT – returned subjects to baseline quicker, perceived soreness more in warm whirlpool
(Montgomery et al., 2008)	n = 9 (M, basketball players)	3-day tournament style basketball competition	Carbohydrate and stretching CWI (11℃, 5x1min) Full leg compression garments	↓sprint and agility (0.7%) ↓vertical jump CWI – maintain acceleration, smaller decrease in sit and reach for CWI
(Rowsell et al., 2009)	n = 20 (M, high-performance soccer players)	4-day simulated soccer match	CWI (10℃), thermoneutral (34℃), counter movement jump height, heart rate, RPE after 5min run and 12x20m repeated sprint, CK 90 min before match, leg soreness 22h after each match	No sig dif ↓ in jump height and repeated sprint ability Lower leg soreness reported in CWI group CK ↑over time – no changes over time

See table 2.3 for list of abbreviations

Table 2.3 continued Summary table of studies demonstrating the effects of different water immersion strategies following exercise induced muscle damage

Authors	Participants	Exercise Protocol	Treatment protocol and measurements	Results
(Rowsell et al., 2011)	n = 20 (M)	Soccer match	CWI (5 x 1 10℃) Thermoneutral (5 x 1 34℃) High-intensity running distance, total distance covered, RPE, leg soreness 22h post match	CWI more effective than thermoneutral for ↓ perception of leg soreness and enhances the restoration of some match-related performance measures
(Vaile et al., 2008b)	n = 38 (M)	Leg press protocol	PAS, CWI, HWI, CWT weighted jump squat, isometric squat, perceived pain, LC – prior, after, 24, 48, 72h post exercise	Squat jump ↑ CWT at 48 and 72h compared with PAS Isometric force ↑ at 24, 48 and 72h following HWI Perceived pain ↑ following CWT at 24, 48 and 72h
(Yanagisawa et al., 2003)	n = 28 (M)	Calf-raises	CON, CWI, double CWI (15min) – magnetic resonance images obtained after, 20, 40 and 60min and 24, 48, 96 and 168h post, ROM, CK, muscle soreness before and after	CON larger ↑in CK at 9h and sig greater muscle soreness at 48h than cooling groups

See table 2.3 for list of abbreviations

Table 2.4 Summary table of studies demonstrating no beneficial effects of different water immersion strategies following exercise induced muscle damage

Authors	Participants	Exercise Protocol	Treatment protocol and measurements	Results
(Jakeman et al., 2009)	n = 18 (F, physically active)	10x10 counter-movement jumps	10min CWI (10°C) CK, perceived soreness, MVC – prior and at 1, 24, 48, 72 and 96h post	No significant group or group x time interactions effects Single bout of CWI shows no beneficial effects on recovery
(King and Duffield, 2009)	n = 10 (F, netball players)	4 session of a simulated netball exercise circuit	PAS, ACT, CWI (9.3±1.6°C) 5 min with 2.5min rest and CWT (9.7±1.4°C [bath, 1min] and 39.1±2.0°C [shower, 2min])	No sig dif evident between groups↓in sprints and vertical jump for CWT and CWI
(Sellwood et al., 2007)	n = 40 (untrained)	5x10 seated leg extension machine – non-dominant leg. 120% of one rep max calculated	Submerged to anterior iliac spines – CWI (5±1°C), CON (24°C) – 3x1min with 1 min rest in between VASP, LC, one leg hop for distance, ISO, CK – baseline, 24, 48 and 72h after	No sig dif between groups – CWI was ineffective in minimising markers of DOMS in untrained individuals

M = male, F= female; CWI = cold water immersion; CON = control; CK = creatine kinase; T_{skin} = skin temperature; MVC = maximal voluntary contraction; PAS = passive recovery; ACT = active recovery; CWT = contrast water therapy; VAS = visual analogue scale of pain; LC = limb circumference; ISO = isometric force contractions; DOMS = delayed onset muscle soreness

Table 2.5 Summary table of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Ascensao et al., 2011)	n = 20 (M, Junior national league footballers	Football match	CWI: 10min 10°C to iliac crest (10) TWI: 10min 35°C (10), immediately post exercise	20m sprint, squat jump, CK, perceived coldness, muscle soreness <0.5, 24, 48h	CWI ↓CK vs. TWI; 24, 48h
(Bailey et al., 2007)	n = 20 (M, habitually active)	90min intermittent simulated football activity	CWI: 10min 10°C to iliac crest (10) CON: seated rest immediately post exercise	Knee extensions, repeated spring, squat jump, CK, body mass, muscle soreness at 0, 1, 24, 48, 168h	CWI ↑MVC 24 and 48h ↑ perceived coldness CWI vs. CON↓ muscles soreness for up to 48h
(Brophy-Williams et al., 2011)	n = 8 (M, well trained team sports)	High intensity interval running (8x3min at 90% $\dot{V}O_{2max}$)	CWI: 15 min in 15°C to midsternal level CON: 15 min seated, 3 CWI performed immediately or 3h post-exercise	Yoyo intermittent, perceived recovery, muscle soreness	CWI post- ↑Yoyo performance CWI 3h post- ↑ Yoyo vs. CON

M = male, F= female; CWI = cold water immersion; CON = control; CK = creatine kinase; T_{skin} = skin temperature; MVC = maximal voluntary contraction; PAS = passive recovery; ACT = active recovery; CWT = contrast water therapy; VAS = visual analogue scale of pain; LC = limb circumference; ISO = isometric force contractions; DOMS = delayed onset muscle soreness, La = lactic acid

Table 2.5 continued Summary of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Coffey et al., 2004)	n = 14 (M)	2 x treadmill runs to exhaustion at 120% and 90% peak running speed – over 4h period	CWT was performed for 15-min after 1 treadmill run – alternating between 60s cold (10℃) and 120s hot (42℃) Passive and active recovery	HR, RPErec, BLa recorded at rest, 4, 8, 12, 16 and 20 min after fist run. Before and after 2nd run, Sprint times	No significant effects between recovery modalities were found. No differences in time or power. ↓RPErec with CWT, ↓BLa recovery with passive
(Costello et al., 2012)	n = 9	Single exercise, 5x20 high-force maximal contractions of left knee extensors	2x3 min in cryochamber at -110℃ (24 and 36h after exercise)	Strength (MVC)	Post 48h: -2.4% Post 72h: -1.3% Post 96h: +4.6%
(Eston and Peters, 1999a)	n = 15(F, University Students)	EF, CONC, ECC, maximal ISO contraction (MVIC) (8x5)	CWI: 15min 15±1℃ exercised arm immersed (n=8) CON	EF MVIC, relaxed are angle, LC, CK, muscle soreness 24, 48, 72h	CWI: ↑relaxed elbow angle ↓CK on days 2 and 3 post-exercise
(Hamlin, 2007)	n = 20 (M; 17, F; 3 development rugby players)	20m multistage shuttle run Repeated sprint test (6x5-, 10- and 15-m sprint shuttles on 30s)	CWI:8-10℃, 1min in seated, 1min standing x 3 CWT:8-10℃, 1min in seated, 1min standing shower 38℃ x 3 CON: 6 min rest	Repeated sprint, ROMS, La – 2h post exercise	CWI; no significant effect on any measure vs. CON CWT; ↓post recovery La CON vs. CWI

See table 2.5 for list of abbreviations

Table 2.5 continued Summary of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Higgins et al., 2011)	n = 26 (M, well trained rugby union)	1 rugby union game and 3 trainings per week for 4 weeks	CWI: 5min in 10-12°C (n=9) CWT: 1 min in 10-12°C 1 min in 38-40°C – alternated cold hot for 7min (n=8) – post games and trainings	Repeated spring, 300-m sprint, perceived degree of rest, tightness, effectiveness of treatment – in week following 4th game	CWI; ↓ repeated sprint performance post-intervention and ↑ tightness 2-days post games vs. CON CWT: ↑ 300-m sprint performance post-intervention and ↑ degree of rest vs. CON
(Howatson et al., 2009)	n = 16 (M, recreationally active)	Drops jumps (5 sets of 20 jumps) repeated 14-21 days later	CWI; 12min in 15±1°C iliac crest (n=8). CON; 12min seated rest – immediately, 24, 48 and 72h post exercise	KE MVIC, mid-thigh girth, CK, muscle soreness, 0, 24, 48, 72, 96h	CWI; no significant effect on any measure vs. CON
(Ingram et al., 2009)	n = 11 (team-sport athletes)	Single exercise 4x20min intermittent sprint, then 20m shuttle run test to exhaustion	Repeated cooling, 2x5min leg CWI at 10°C(2.5min break) applied 0 and 24h after exercise, CWT and CWI	Sprint (10x20m) Strength (MVC) Blood samples and muscle soreness ratings before and after, 24h and 4bh post exercise - ↑ return to baseline. ↓ in muscle soreness at 24h	CWI resulted in significantly lower muscle soreness rating, ↓ isometric leg extensions and flexion strength @ 48h.

See table 2.5 for list of abbreviations

Table 2.5 continued Summery of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Jakeman et al., 2009)	n = 18 (F, physically active)	CMJ (10x10 jumps)	CWI:10min in 10±1℃ seated to superior iliac crest – 10min post exercise	Quadriceps MVIC, CK, limb soreness @ 1, 24, 28,72, 96h	CWI no significant effect on any measures vs. CON
(King and Duffield, 2009)	n = 10 (F, netball players active 4-5x a week)	Single exercise 4x15min intermittent sprints	2x5min leg CWI at 9.3℃ (2.5min break)	Sprint (5x20m) Jump	No significant differences between conditions for vertical jump, 20m + 10m spring. ↓ lactate post intervention
(Kuligowski et al., 1998)	n = 56 (M; 28, F; 28, University students)	Non-dominant arm elbow flexor eccentric contractions (5x10 at > IRM concentric)	CWI; 24min in 13℃. Exercised arm immersion to mid-deltoid (n=14) HWI; 24min in 39		
Minett et al., 2012)	n = 8 (M, team-sport athletes)	2x35min bout of intermittent-sprint shuttle running. 15min recovery	20min or 10min cooling with towel, cooling vest and cooling packs	Sprints, shuttle running distance covered, MVC, voluntary activation (VA) pre- post- intervention and mid- post- exercise, blood via venous blood draws	↑shuttle-running distance covered follow 20min cold application.

See table 2.5 for list of abbreviations

Table 2.5 continued Summary of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Parouty et al., 2010)	n = 10 (M - 5, F – 5, national level swimmers)	100m freestyle swimming time trial, repeated 30min later	CWI; 5min in 14-15℃ to shoulders seated CON; 5min seated rest – 5min post-exercise	100m freestyle swimming, HR peak, change and variability, La, perceived recovery, 30min	CWI; ↓ swim time, HR change and peak vs. CON ↑ perception of recovery vs. CON
(Peiffer et al., 2010)	n = 10 (M, well trained cyclists)	25min of cycling at 65% $\dot{V}O_{2max}$ then a 4km time trial in 35℃, repeated 15min later	CWI: 5min in 14℃ seated to midsternal level CON; 15min seated rest in 15℃ – 5min post-exercise	4km time trial time, average power, VO_{2max} and economy, 15min	CWI; ↑ time-trial 2 and average power
(Pointon and Duffield, 2012)	n = 10 (M, team-sport athletes)	2x30min intermittent sprint exercise in 32℃ and 52% humidity – tackling or no tackling	20min CWI (8.9 ± 0.9℃) or passive recovery	Sprint time and distance covered, MVC, VAS, blood markers before, after, after, 2 and 24h post recovery	↑MVC in CWI immediately after recovery. No effect on blood markers ↓VASP with passive recovery
(Pointon et al., 2012)	n = 10 (M, team-sport athletes)	2x30min intermittent sprint exercise in 32℃ and 52% humidity	20min CWI (8.9 ± 0.9℃) or passive recovery	Perceived muscle soreness and pain, blood markers – pre- and post- exercise, post recovery, 2, 24 and 48h post exercise	↓ MVC in below pre-exercise values for 24h recovery ↑ blood markers post exercise

See table 2.5 for list of abbreviations

Table 2.5 continued Summary of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Pointon et al., 2011)	n = 10 (M, resistance-trained rugby players)	2 x 25 maximal concentric/ECC muscle contractions dominant knee extensors	20min recovery (CWI c CON)	Perceived muscle soreness and pain, blood markers – pre- and post- exercise, post recovery, 2, 24 and 48h post exercise	↓ voluntary torque, ↑ perceived muscle soreness and muscle damage markers. No sig ↑ in MVC in CWI. No sig dif in CK between groups
(Robey et al., 2009)	n = 20 (M 12, F 8m club and junior-state rowers)	Stair climbing and descending running task	CWT; 2 min in 40°C shower, 1 min in 12°C water waist deep, 5 x 15 static stretching CON: 15 min seated rest – immediately, 24, 48h post	Rowing 2km ergometer maximal concentric leg extension isokinetic torque – 72h CK, muscle soreness - 0, 24, 48h	CWT; no significant effect on any performance versus CON
(Rowsell et al., 2011)	n = 13(M, under 17 state level footballers	Football tournament containing 1 match per day for 4 days	CWI; 1 min in 10°C to midsternal level, 1 min out of water seated x5 (n=6) TWI; 1min in 34°C to midsternal level, 1 min out of water seated x 5 (n=7)	football match total time, high intensity running distance, time in HR zones, leg soreness, perceived leg fatigue, RPE	CWI; ↑ total running distance and time in moderate HR zones, ↓ leg soreness and general fatigue vs. TWI

See table 2.5 for list of abbreviations

Table 2.5 continued Summary of studies demonstrating CWI, CWT and HWI as a form of recovery intervention

Authors	Participants	Exercise Protocol	Interventions	Measures and time points	Results
(Vaile et al., 2008b)	n = 38 (M, strength trained)	7x10 eccentric repetitions on leg press machine	PAS, hydrotherapy – CWI (15℃), HWI (38℃) CWT; alternate between cold and hot – 24 hour interval for 72h post exercise for 14min	Squat jump, CK, thigh circumference, VASP pre- and post- exercise, 24, 48 and 7h post- exercise	No difference between any interventions at baseline or immediately post exercise ↑squat at 24, 48 and 7h post- exercise following HWI and CWT ↓CK 24h and 72h
(Vaile et al., 2008a)	n = 10 (M, well trained cyclists)	5 x30min combined intermittent sprint and time trial. $\dot{V}O_{2peak}$ test on cycle ergometer	Intermittent CWI in 10, 15 and 20℃, continuous CWI in 20℃ or active recovery or passive recovery (40min)	Ratings of perceived exertion, total work, core, skin temp, BLa, HR	No sig dif in total work observed between CWI protocols
(Sellwood et al., 2007)	n = 40 (M 11, F 29, untrained adults)	Non-dominant eccentric leg extensions (5x10 at 120% of concentric 1RM)	CWI; 1 min in 5±1℃ to anterior superior iliac, standing. 1 min out of water x 3 TWI; 1 min in 24±1℃ – post exercise	Quadriceps MVIS 1-legged hop for distance, LC, CK, algometer pressure, leg soreness, perceived leg soreness – 24, 48, 72h	CWI; no significant effect on any measure vs. TWI

See table 2.5 for list of abbreviations

2.4.3 Summary

Overall, it is apparent that there are some beneficial effects of CWI, HWI, and CWT on alleviating the signs and symptoms of EIMD. With the array of different temperatures, depths, and duration of water immersion employed, and the different types of exercise used to induce muscle damage it is difficult to determine the optimal method of attenuating the effects of eccentric exercise on the signs and symptoms of EIMD, and subsequently performance. Additionally, it is important to consider that many studies use isolated muscles (Howatson & Someren, 2008) to establish the effects of recovery methods on EIMD, which may not emulate well with whole body exercise. Furthermore, the majority of the studies use untrained participants unaccustomed to muscle damaging exercise, and therefore, may not respond to the muscle damaging exercise and recovery intervention in a similar manner to trained individuals. Trained individuals may be accustomed to eccentric exercise due to the repeated bout effect (Nosaka et al., 1991, Nosaka et al, 2001) and therefore could respond to recovery strategies differently. Untrained individuals however, may not be accustomed to eccentric exercise, leading to different responses to recovery strategies. However, recreationally trained and untrained individuals who engage in unaccustomed eccentric exercise may experience EIMD and the associated signs and symptoms and subsequently a decrease in overall performance. Therefore, it is also important for these individuals to recover from EIMD allowing an increase in exercise adherence. For this study, non-trained instead of trained individuals were used to ensure that the muscle damaging protocol produced a sufficient amount of muscle damage to be able to observe clear decrements in the indirect markers used in the study. Non-trained individuals may also be more susceptible to muscle damage post

exercise and may benefit more from recovery interventions, where as trained individuals may be accustomed to an exercise protocol and may only benefit from recovery interventions during a match/tournament over several games/days/weeks. Non-trained individuals were also easily accessible as university students were targeted for this study.

Despite the confounding issues surrounding the controversial effect of water immersion on the signs and symptoms of EIMD, there is evidence that water immersion may attenuate the performance decrement associated with EIMD. However, it is presently unclear as to whether HWI and CWT, which may be more ecologically valid and better tolerated by individuals compared to CWI, can alleviate the signs and symptoms of EIMD, and therefore, attenuate the decrement in performance immediately following muscle damaging exercise.

Finally, the mechanisms surrounding the effects of CWT, and HWI and the causations of EIMD, DOMS and the accompanying signs and symptoms remains equivocal. Therefore, further research into the effects of CWT and HWI over a time period in which the signs and symptoms of EIMD have been shown to reduce an athlete or individual's performance is required.

Chapter 3:

General Methods

This chapter provides detailed explanation of the general procedures and methods used to collect data within this thesis. Specific methods are described in each methodological section within study 1 and study 2.

3.1 Participants

Eighteen healthy, physically active male participants (mean ± SD: height 177.8 ± 7.2 cm; body mass 74.3 ± 8.5 kg; age 21 ± 2 years) from the University of Bedfordshire volunteered to participate in this study. Prior to testing participants were clearly informed of the potential risks and discomforts of the tests (see Appendix 1), and were asked to read and sign an informed consent form, a Physical Activity Readiness Questionnaire (PAR-Q), Medical Health Questionnaire, and a Blood Analysis Screening Form (see Appendix 2 - 5). All participants completed functional and performance measures (limb circumference of the rectus femoris, gastrocnemius, and bicep femoris, pressure pain threshold of the rectus femoris, gastrocnemius, and bicep femoris, CK, VAS, range of motion, squat jump, and flexibility) on every visit to the laboratory.

To avoid the protective effects of muscle damaging exercise, participants who had performed any resistance training in the previous 6 months (McHugh et al., 1999, Nosaka et al., 2001) were excluded from the study. Additionally, individuals who regularly took pain killers and anti-inflammatory medication were excluded from the study as non-steroidal anti-inflammatory medication may be effective in attenuating muscle dysfunction and soreness (Baldwin Lanier, 2003, Trappe et al., 2002, Nieman et al., 2005). Participants were asked not to engage in any sporting activities

throughout the testing period to ensure muscle damage occurred from the muscle damaging exercise protocol only. Furthermore, for the duration of the study participants were asked to refrain from changing their normal eating and dietary habit (Chen et al., 2011b), repress any nutrition supplements (Jakeman et al., 2010), avoid alcohol or drug intake (Chen et al., 2011a, Chen et al., 2011b), avoid saunas (Hausswirth et al., 2011), hot or cold baths, avoid the use of compression garments (Jakeman et al., 2010), and any additional exercise before and during the testing period of 7-14 days. Ethical Approval was granted from the Institute of Sport and Physical Activity Research Ethics panel at the University of Bedfordshire. All testing procedures were conducted within the Sport and Exercise Science Laboratory at the University of Bedfordshire (Temperature 18 - 23℃ and relative humidity between 45.5 – 55%). Participants were free to withdraw from the study at any point without question, and confidentiality and anonymity were ensured. Participant's training status was not measured for this study. Participants were randomly assigned to each group and therefore an individual's training status is relative to that specific individual.

3.2 Indirect Markers of Muscle Damage

3.2.1 Assessment of Range of Motion

Range of motion is commonly used as indirect evidence of muscle damage (Clarkson et al., 1992, Byrnes et al., 1985, Clarkson & Tremblay, 1988, Ebbeling & Clarkson, 1990, Howell et al., 1985, Newham et al, 1983, Nosaka & Clarkson, 1992). ROM of the knee angle (during flexion) of the dominant and non-dominant leg was determined using a goniometer (Baseline 8" Plastic 360 Degree Goniometer). For the

measurement of knee flexion, participants lay prone on a plinth with both knees fully extended. From this position participants were asked to fully flex their knee as much as possible without force or using their hands to assist the movement, to the point without pain. Anatomical landmarks (lateral epicondyle of the femur, lateral malleous and greater trochanter) were used to ensure proper alignment (Tokmakidis et al., 2003, Howatson and van Someren, 2008). Landmarks were marked with a semi-permanent pen to ensure consistency on subsequent measure (Howatson and van Someren, 2008). The measurement was taken 5 times on each leg and the average was reported.

3.2.2 Assessment of Limb Circumference and Skin Temperature

Limb circumference measurements were taken as an indicator of acute changes in thigh volume (Eston and Peters, 1999b) likely to occur from osmotic fluid shifts or inflammation, which is often been associated with muscle-damage and eccentric exercise (Vaile et al., 2008b). Mid-thigh (belly of rectus femoris) was selected for representation of anterior leg measurements as it closely resembled changes throughout the entire upper leg (Vaile et al., 2008b). Limb circumference was measured at three different points of each leg using a non-stretch anthropometric measuring tape while the participants lay supine with their heel on the plinth and their knee slightly flexed. This position made it easier to determine the thickest part around the upper thigh and calf, which indicates the muscle belly of the superficial muscles. Upon contractions, the muscle belly becomes shorter and thicker and in superficial muscles, this change can be observed on the surface (Scheepers et al., 1997). Participants were required to contract and then relax, with the belly of the muscle noted during the contraction phase. Measurements were taken from the largest part

around the lower leg and the upper thigh, the third measurement was taken at the half way point from the lateral head of the patella and the largest part around the upper leg. Measurements were taken at the same site on every occasion which was marked using a semi-permanent ink pen (Chen et al., 2007a, Chen et al., 2011b). This was to ensure re-test reliability (Vaile et al., 2008b).

Skin temperature (T_{skin}) measured using skin thermistors (Grant, EUS-U-VS5-0, Dorset, United Kingdom) which were applied to the gastrocnemius (lateral head), bicep femoris, and rectus femoris of each leg were obtained to ensure that any changes in limb circumference was not due to changes in temperature. Participants stood in the correct anatomical position and the skin thermistors were applied to the belly of the muscle which was determined by contracting the specific muscle, which then becomes shorted and thicker (Scheepers et al., 1997). Measurements were taken from the largest part of the surface area of the gastrocnemius and the upper thigh, the third measurement was taken from the largest surface area of the bicep femoris. Measurements were taken at the same site on every occasion which was marked using a semi-permanent ink pen (Chen et al., 2007a, Chen et al., 2011b). This was to ensure re-test reliability (Vaile et al., 2008b).

3.2.3 Assessment of Delayed Onset Muscle Soreness

Pressure pain threshold (PPT) was measured using an Algometer (Wagner, FDK40, Wagner Instruments) as it is a popular method of assessing perceived pain that is both reliable and valid (Cook et al., 1997). Pressure pain threshold was defined to the participant as the ‘point at which the pressure sensation just becomes painful’ (Gibson

et al., 2006). Participants were instructed to go just beyond the point of being uncomfortable; 'uncomfortably uncomfortable'. The round force dial of the algometer tip had an area of $1cm^2$ and was placed onto the belly of the muscle at three separate points; rectus femoris, bicep femoris, and gastrocnemius. For the measurements of the bicep femoris and gastrocnemius participants were required to lie prone with their feet hanging over the plinth. This allowed the gastrocnemius to be in a relaxed position, to allow the full force of the algometer to be applied without pressure on the ankles. The measurements were taken at the same point on every occasion which was indicated using a semi-permanent skin marker (Chen et al., 2007a, Chen et al., 2011b). Force was gradually applied until the participant reported the first feeling of noticeable pain. Subjects were familiarised with 5 seconds of pressure in no-pain areas before the assessments during the familiarisation stage.

3.2.4 Assessment of Neuromuscular Function

Neuromuscular function was measured using a squat jump (Jump Mats, Probiotics, Just Jump System, Cranlea) in order to measure power. Squat jumps have been used extensively in the muscle damaging literature to measure recovery and neuromuscular function following EIMD (Howatson et al., 2009, Howatson & Milak, 2009, Pournot et al., 2011), and may be a practical tool for practitioners to use within a field setting (Markovic et al., 2004). Participants were instructed to begin in an upright position, with their feet shoulder width apart. Participants then performed a full-squat (to approximately a 90^0 knee angle (Howatson et al., 2012)) with their hands crossed over their chest (this was to prevent the participant from using their arms to assist the jump) and jump vertically, keeping their hands folded across their chest and ensuring they

kept their legs straight. Participants completed five full jumps in order to obtain an average. Participants were allowed a 30 second rest in between each jump, to allow time to recover. Each participant jumped 5 times and a mean was reported (measured to 2 decimal places). Participants did not warm up prior to performing squat jumps as a dynamic warm up has been shown to enhance jump performance as a warm up can help prepare the inactive muscle for power performances (Dixon et al., 2010).

3.2.5 Assessment of Flexibility

Participant's flexibility was assessed using the simple Sit & Reach Bench (Cranlea, Medical Electronics) method. Flexibility is widely used as a measurement tools to assess hamstring and lower back flexibility (Ayala et al., 2012). With the increase in stiffness after muscle damage exercise, this method is commonly used to assess lower body flexibility (Mier, 2011). The sit and reach test was performed using a sit and reach box with a height of 33 cm. The participants were instructed to place the soles of both feet flat against (90^0 dorsiflexion, (Ayala et al., 2012)) the sit and reach box with their legs fully extended. Several variables have been reported to affect the reproducibility of hamstring flexibility, differences in pelvic positions and stability, ankle position (dorsiflexion vs. plantar flexion) (Ayala et al., 2012). Hands were positioned with one hand on top of the other whilst arms were held straight out in front at shoulder height. The participant was instructed to take a deep breath out while leaning forward as far as possible without bending their knees. Participants were instructed to hold their final position for 2 seconds. Participants were asked to stretch to the point just beyond being uncomfortable. Participants performed the measure five times, with 30 second rest in between each movement, to gain an average (value was

recorded to the nearest cm). A reach distance of 15 cm corresponded to the position of the feet against the box.

3.2.6 Assessment of Creatine Kinase

Blood samples were obtained during familiarisation, pre- and 2 min post- downhill (PRE), and again at 24, 48 and 72 h post downhill run. Blood was obtained using standard finger prick blood collection techniques and immediately analysed for CK using universal Reflotron (Reflotron Plus Blood Analyser, Roche, Una Health) blood analysis techniques.

3.2.7 Statistical Analysis

Statistical analyses were conducted using the statistical package IBM SPSS Statistics version 19 (SPSS Inc, Chicago, IL, USA). Prior to inferential statistical analyses, descriptive tables were generated to check the central tendency and dispersion of the data. Quantile-quantile (Q-Q) plots were generated to infer if the normality assumption of the inferential tests were met. The instances in which the normality assumptions were not met, the relevant data was log-transformed. If the normality assumption was still not met, non-parametric inferential tests were used.

Corresponding data analysis for each experimental chapter, according to aims, will be discussed in relevant methodology sections of each chapter.

Chapter 4:

Experimental Study 1

4.1 Introduction

Reliability refers to the reproducibility of a measurement tool in repeated trials on the same individual (Hopkins, 2000, Currell and Jeukendrup, 2008, Atkinson and Nevill, 1998), and is defined as the consistency of measurement (Watkins et al., 1991). There is considerable interest within sports science and sports medicine regarding the

phenomenon of eccentric based EIMD (Warren et al., 1999). Subsequently, there is a wide range of measurement tools employed to quantify the magnitude and duration of the signs and symptoms associated with EIMD and the validity of these measurement tools are dependent on the reliability of that tool (Warren et al., 1999).

Reliability testing is an important measure in sport and exercise performance, research, and intervention studies as it highlights any systematic bias, and inherent typical error (e.g. technical or biological variation) associated with a particular measurement (Currell and Jeukendrup, 2008). Subsequently, quantification of this error allows scientists, researchers, and coaches to be confident that any significant changes across time are due to the intervention and not due to random variability. Furthermore, an important consideration for researchers and practitioners is to select a measure that produces the greatest reliability and validity, with the least amount of equipment and preparation time (Hopkins, 2000). Therefore, this information needs to be readily available. However, there is a scarcity of literature that demonstrates the reproducibility of the assessment of markers of muscle damage. Typically, improved reliability can help with the precision of a single measurement as well as recognise changes within the measurements between trials. In turn, this ensures systematic changes in the mean of a measures between two consecutive trials does not represent learning effects, motivation or fatigue (Hopkins, 2000).

Realistically, there will always be some degree of variation between two or more continuous measurements. Therefore, the degree of acceptable reliability needs to be established for the effective use of a measurement tool (A tkinson and Nevill, 1998). For example, the reliability of maximal voluntary contraction measurements is high

(ICC > 0.85), whilst even though the reliability of range of motion measures are reported to be reliable, with ICC's ranging from 0.78 to 0.97 (Jakobsen et al., 2010), they have not been well documented (Warren et al., 1999). One study demonstrated an ICC of 0.92 for flexibility testing using the sit and reach method (Ayala et al., 2012), indicating high reproducibility when comparing other methods of assessing flexibility (ICC < 0.89 for toe touch test and passive straight leg raise test). ICC for muscle soreness using VAS appears to be high, indicating good reliability (Bijur et al., 2001) as well as excellent intra-rater reliability for PPT using a standard algometer (Cathcart and Pritchard, 2006). There is a large variability in CK response to exercise that is not precisely understood (Warren et al., 1999). However, several studies have reported high reliability in CK after eccentric exercise (Newton et al., 2008, Chen et al., 2011). A CV of 10.2% and an ICC of 0.84 was reported in Chen and colleagues 2011 study (Chen et al., 2011) and an ICC of 0.93 was reported in Newton and colleagues 2008 study (Newton et al., 2008), with both studies demonstrating high reliability for CK. The differences between some studies reporting low reliability and other studies report high reliability may be due to training status between the participants, genetics and the variations in the time period between trials.

It is important to determine the reliability of indirect markers of muscle damage in order to assess the effects of eccentric-based exercise muscle damage on recovery and subsequent performance. The indirect markers of muscle damage allow researchers, trainers and athletes the chance to monitor the rate of recovery and the effects of performance after muscle damaging exercise. However, it is ideal that these indirect markers have a sufficient level of reproducibility in order to avoid systematic bias and random error due to biological or mechanical variation (Atkinson and Nevill, 1998).

If the indirect markers of muscle damage produce minimal variation between several different time points there can be an improvement of the precision of separate measurements and allow for better observation of the changes in values over several different time points (Hopkins, 2000).

4.2 Methods

4.2.1 Participants

Eighteen healthy, physically active male participants (mean ± SD height 177.8 ± 7.2 cm; body mass 74.3 ± 8.5 kg; age 21 ± 2 years) volunteered for this study. All participants completed necessary documentation and pre-test guidelines outlined in Chapter 3, section 1 (3.1).

4.2.2 Experimental Design

Participants visited the laboratory on two separate occasions separated by a minimum of 4 days and a maximum of 7 days. During visit 1 and visit 2, identical measures, in a specific order, were taken during each visit. Participants were required to wear lose fitting shorts and a t-shirt, and refrain from changing their running shoes for each visit. Temperature and humidity in the laboratory ranged between 18 – 22^{0}C and 45.5 – 55%

respectively. During visit 1, participant's anthropometric measurements were collected (see section 3.2).

4.2.3 Indirect Markers of EIMD

Indirect markers of EIMD include limb circumference (rectus femoris, gastrocnemius, and bicep femoris), pressure pain threshold (rectus femoris, gastrocnemius, and bicep femoris), CK, perceived muscle soreness (VAS), ROM, squat jump, and flexibility. Measurements were taken during visit 1 and visit 2 as outlined in the general methodology (see section 3.3).

4.2.4 Statistical Analysis

Dependent T-tests were used to check there were no significant difference between trial 1 and trial 2. Normality of the observed data was assessed using Q - Q plots and deemed plausible in all instances accept for CK. Subsequently, CK was logarithmic transformed and the statistical analysis was performed using the logarithmic transformed variables. For CK, raw data was reported. The typical error of measurement; The coefficient of variation (CV), which is expressed as a percentage, was calculated by dividing the standard deviation of the difference by the square root of two (1.414) and dividing the answer by the grand mean (Hopkins, 2000). The CV was used to determine within subjects reliability. A CV of <10% has been deemed to represent good reliability (Atkinson and Nevill, 1998, Stokes, 1985) and 10-15% as acceptable reliability (Brughelli and Van Leemputte, 2013). 95% limit of agreement (LoA) was also used to measure the test re-test reliability (Atkinson and Nevill, 1998).

95% LoA will be accompanied by Bland-Altman plots in order to schematically show the measurement error (Atkinson and Nevill, 1998). ICC was used to determine the between day reliability, in other words the repeatability (Brughelli and Van Leemputte, 2013) as well as to determine the degree to which individuals maintained their position within each group (trial 1 and trial 2) upon retesting. An ICC close to 1 indicates 'excellent' reproducibility, therefore, an ICC of above 0.90 is deemed as 'high' reliability, values between 0.80 and 0.89 is considered 'good' reliability and values lower than 0.80 is deemed 'questionable' reliability (Atkinson and Nevill, 1998). However, ICC is a limited measure of reliability due to the influence of the magnitude of the between-subject variation and it does not indicate possible systematic bias (Ayala et al., 2012). Data are present as mean ± SD, and significance was accepted as $p < 0.05$.

4.3 Results

The results from the indirect markers of EIMD are shown in Tables 4.1 – 4.4. Dependent T-tests indicated no systematic bias ($p \geq 0.05$) between trial 1 and trial 2 except for CK ($p = 0.02$) (Table 4.1).

Table 4.1 Values obtained from the indirect markers of EIMD (CK, squat jump, flexibility, ROM of the left leg, ROM of the right leg and VAS) during trial 1 and trial 2

Measure	Trial 1			Trial 2			Trial 1- trial 2 differences			
	Mean	SD	Range	Mean	SD	Range	Mean diff	95% CI	SD	p value
CK (IU/L)	464.87	623.65	24 – 2580	255.55	146.57	97 - 685	239.28	-80.66, 559.21	523.08	0.02
SJ (cm)	45.53	5.61	21.40 - 37.40	44.7	5.67	17.54 - 36.56	0.83	-0.85, 2.52	3.40	0.87
Flexibility (cm)	16.95	7.66	6.20-31.80	17.07	7.67	4.20 - 31.0	-0.12	-2.18, 1.94	0.97	0.98
ROM L (^{0})	59.97	5.73	48 - 69.20	59.64	6.39	45.00 - 73.00	-0.03	-1.71, 2.38	4.12	1.00
ROM R (^{0})	58.42	4.78	48.80 – 67	58.33	6.27	44.40- 70.20	-0.09	-2.22	2.39	0.36
VAS	1.56	1.42	0 – 4	2.78	1.26	1 – 5	1.22	-2.16 -0.27	1.89	0.39

Mean diff = mean difference between trial 1 and trial 2; 95% CI = lower and upper bounds of the 95% confidence interval for the mean difference; SD = standard deviation of trial 1 and trial 2 respectively; p value = significant difference between trial 1 and trial 2 for each variable; CK = creatine kinase; SJ = Squat jump; ROM L = range of motion of the left leg; ROM R = range of motion of the right leg; cm = centimetre; mm = millimetre. For descriptive purposes raw data was reported for all variables. CK values were reported with raw data.

Table 4.2 Values obtained during trial 1 and trial 2 for left and right leg limb circumference (cm)

Measure	Trial 1			Trial 2			Trial 1- trial 2 differences			
	Mean	SD	Range	Mean	SD	Range	Mean diff	95% CI	SD	p value
LC_L_RF	48.91	2.93	44.00 - 54.00	48.81	3.54	44.0 - 56.0	-0.11	-0.99, 1.21	2.23	0.59
LC_L_GN	54.96	4.12	43.10 - 61.00	54.98	3.67	50.0 - 62.00	0.02	-1.46, 1.42	2.90	0.97
LC_L_BF	37.45	1.56	35.00 - 40.60	38.83	4.49	34.00 - 55.00	1.38	-3.61, 0.84	4.48	0.15
LC_R_RF	54.96	4.55	43.10 - 62.00	55.14	3.52	50.0 - 61.0	0.18	-1.96, 1.61	3.59	0.44
LC_R_GN	38.01	2.60	35.00 - 44.10	37.69	2.30	34.4 - 43.0	-0.32	-0.05, 0.70	0.76	0.86
LC_R_BF	48.48	3.09	43.00 - 55.00	47.93	4.15	43.0 - 60.0	-0.55	-1.16, 2.26	3.44	0.62

Units; cm = centimetre; LC = limb circumference; L = left leg; R= right leg; RF = rectus femoris; GN = gastrocnemius; BF = bicep femoris; see footnote of Table 4.1 for descriptions of abbreviations not listed

Table 4.3 Values obtained during trial 1 and trial 2 for left and right leg pressure pain threshold (KgF)

Measure	Trial 1			Trial 2			Trial 1- trial 2 differences			
	Mean	SD	Range	Mean	SD	Range	Mean diff	95% CI	SD	p value
PPT_L_RF	6.29	1.83	3.20 - 9.20	6.57	1.27	4.20 - 8.40	0.28	-1.33, 0.78	2.13	0.21
PPT_L_GN	6.58	1.58	4.40 - 9.20	7.36	2.10	4.00 - 13.00	0.78	-1.80, 0.24	2.06	0.56
PPT_L_BF	7.47	1.46	5.00, 10.00	1.94	1.49	5.00 - 11.20	0.48	1.56, 0.61	2.19	0.56
PPT_R_RF	6.79	2.01	4.40 - 12.00	6.92	1.43	3.6 - 9.4	0.13	-1.16, 0.89	2.08	0.38
PPT_R_GN	6.99	1.67	4.40 - 9.60	7.57	1.80	4.8 - 11.6	0.51	-1.39, 0.38	1.73	0.28
PPT_R_BF	7.05	1.74	4.80 - 10.60	7.83	1.89	3.0 - 10.8	0.78	-1.86, 0.31	2.19	0.86

Units; KgF = kilogram of force; PPT = pressure pain threshold; L = left leg; R= right leg; RF = rectus femoris; GN = gastrocnemius; BF = bicep femoris; see footnote of Table 4.1 for descriptions of abbreviations not listed

Tables 4.4 – 4.6 demonstrates the reproducibility statistics for responses to the indirect markers of EIMD (CK, squat jump, flexibility, ROM of the left leg, ROM of the right leg and VAS, limb circumference, and pain pressure threshold) during trial 1 and trial 2. The CV (%), the ICC and the limits of agreement are presented. After each table, Bland-Altman graphs are shown corresponding to the values obtained for the indirect markers of EIMD.

Table 4.4 Reproducibility statistics for the indirect markers of EIMD (CK, squat jump, flexibility, ROM of the left leg, ROM of the right leg and VAS) during trial 1 and trial 2

Measure	CV (%)	ICC	95% LoA
CK (IU/L)	116.0	0.19	239.28 + 1.96 x 523.02 = 1264.40 239.28 - 1.96 x 523.02 = -785.84
SJ (cm)	5.3	0.81	0.83 + 1.96 x 3.40 = 6.79 0.83 – 1.96 x 3.40 = - 5.83
Flexibility (cm)	17.4	0.85	-0.122 + 1.96 x 0.97 = 1.78 -0.122 – 1.96 x 0.97 = -2.02
ROM L (cm)	4.8	0.76	-0.03 + 1.06 x 4.12 = 8.05 -0.03 – 1.96 x 4.12 = -8.12
ROM R (cm)	5.5	0.65	-0.09 +1.96 x 2.39 = 4..59 -0.09 – 1.96 x 2.39 = -4.77
VAS	61.0	0.01	1.22 + 1.96 x 1.89 = 4.92 1.22 – 1.96 x 1.89 = -2.48

CV = coefficient of variance; ICC = intraclass correlation; 95% LoA = 95% limits of agreement; See footnote of Table 4.1 for abbreviations not listed

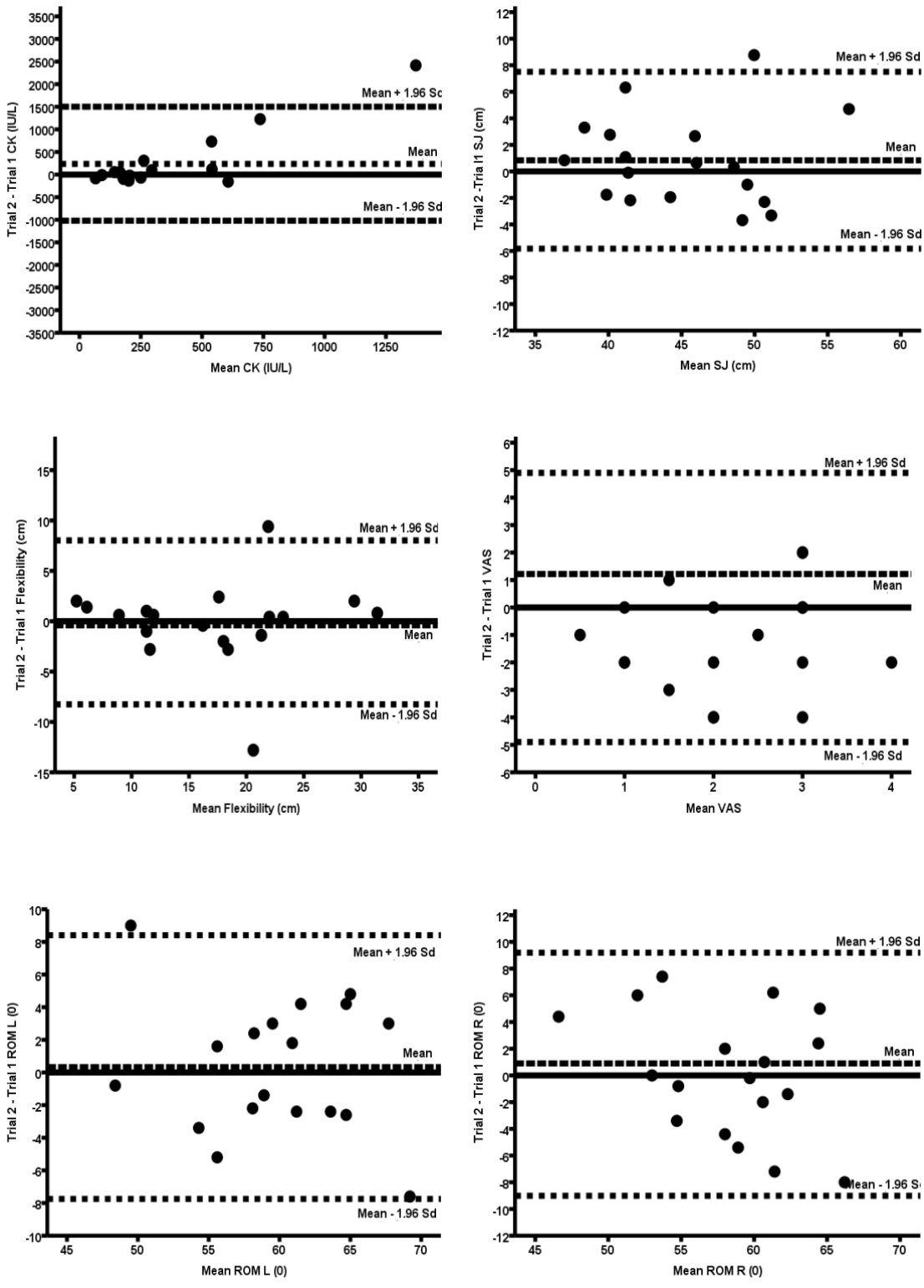

Figure 4.1 Bland-Altman plots showing difference between trial 1 and trial 2 in relation of the mean of the two trials for each individual for CK (IU/L), SJ (cm), ROM L (^{0}), ROM R (^{0}), Flexibility (cm) and VAS. Dashed lines are plotted indicating the 95% limits of agreement using mean difference ± 1.96 x SD of difference

Table 4.5 Reproducibility statistics for limb circumference of the left and right leg during trial 1 and trial 2.Units; cm

Measure	CV (%)	ICC	95% LoA
LC_L_RF	3.2	0.77	-0.11 + 1.96 x 2.23 = 4.26 -0.11 – 1.96 x 2.23 = -4.40
LC_L_GN	3.7	0.72	0.02 + 1.96 x 2.90 = 5.70 0.02 - 1.96 x 2.90 = -5.66
LC_L_BF	8.4	0.11	1.38 + 1.96 x 4.48 = 10.16 1.38 - 1.96 x 4.48 = -7.40
LC_R_RF	4.6	0.61	0.18 + 1.96 x 3.59 = 7.22 0.18 - 1.96 x 3.59 = -6.86
LC_R_GN	1.5	0.95	-0.32 + 1.96 x 0.76 = 1.17 -0.32 - 1.96 x 0.76 = -1.81
LC_R_BF	5.0	0.56	-0.55 + 1.96 x 3.44 = 6.19 -0.55 - 1.96 x 3.44 = -7.29

CV = coefficient of variance; ICC = intraclass correlation; 95% LoA = 95% limits of agreement; See footnote of Table 4.2 for abbreviations not listed

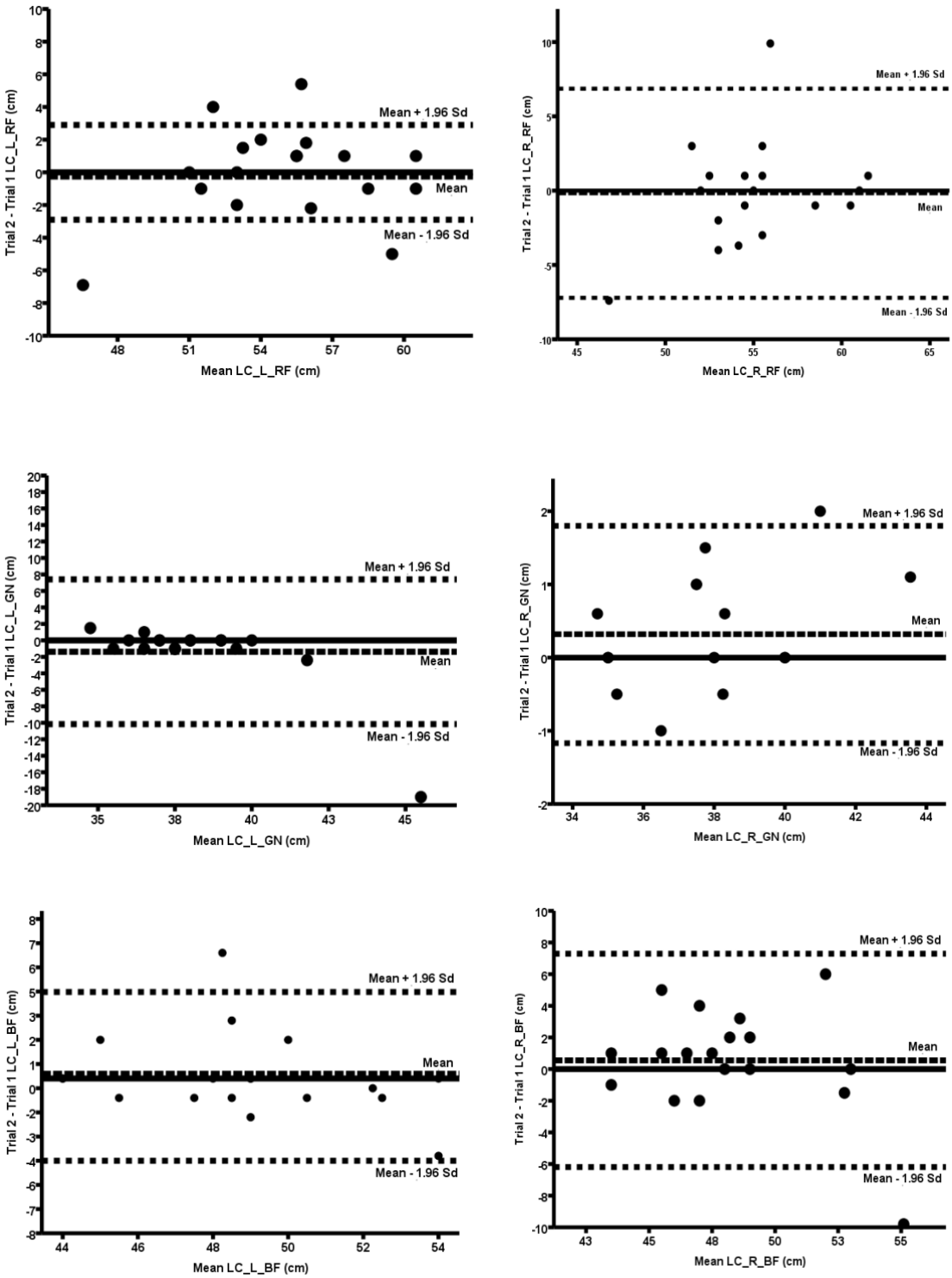

Figure 4.2 Bland-Altman plots showing difference between trial 1 and trial 2 in relation of the mean of the two trials for each individual for limb circumference (cm) of the left rectus femoris, gastrocnemius and bicep femoris and right rectus femoris, gastrocnemius and bicep femoris. Dashed lines are plotted indicating the 95% limits of agreement using mean difference ± 1.96 x SD of difference

Table 4.6 Reproducibility statistics for PPT of the left and right leg during trial 1 and trial 2. Units; KgF (kilograms of force)

Measure	CV (%)	ICC	95% LoA
PPT_L_RF	23.2	0.08	0.28 + 1.96 x 2.13 = 4.20 0.28 - 1.96 x 2.13 = -3.89
PPT_L_GN	21.2	0.38	0.78 + 1.96 x 2.06 = 4.82 0.78 - 1.96 x 2.06 = - 3.25
PPT_L_BF	20.2	-0.10	0.48 + 1.96 x 2.19 = 4.77 0.48 - 1.96 x 2.19 = -3.81
PPT_R_RF	21.5	0.29	0.13 + 1.96 x 2.08 = 4.21 0.13 - 1.96 x 2.08 = -3.95
PPT_R_GN	16.4	0.51	0.51 + 1.96 x 1.73 = 3.90 0.51 - 1.96 x 1.73 = -2.88
PPT_R_BF	21.0	0.27	0.78 + 1.96 x 2.19 = 5.07 0.78 - 1.96 x 2.19 = -3.51

CV = coefficient of variance; ICC = intraclass correlation; 95% LoA = 95% limits of agreement; See footnote of Table 4.3 for abbreviations not listed

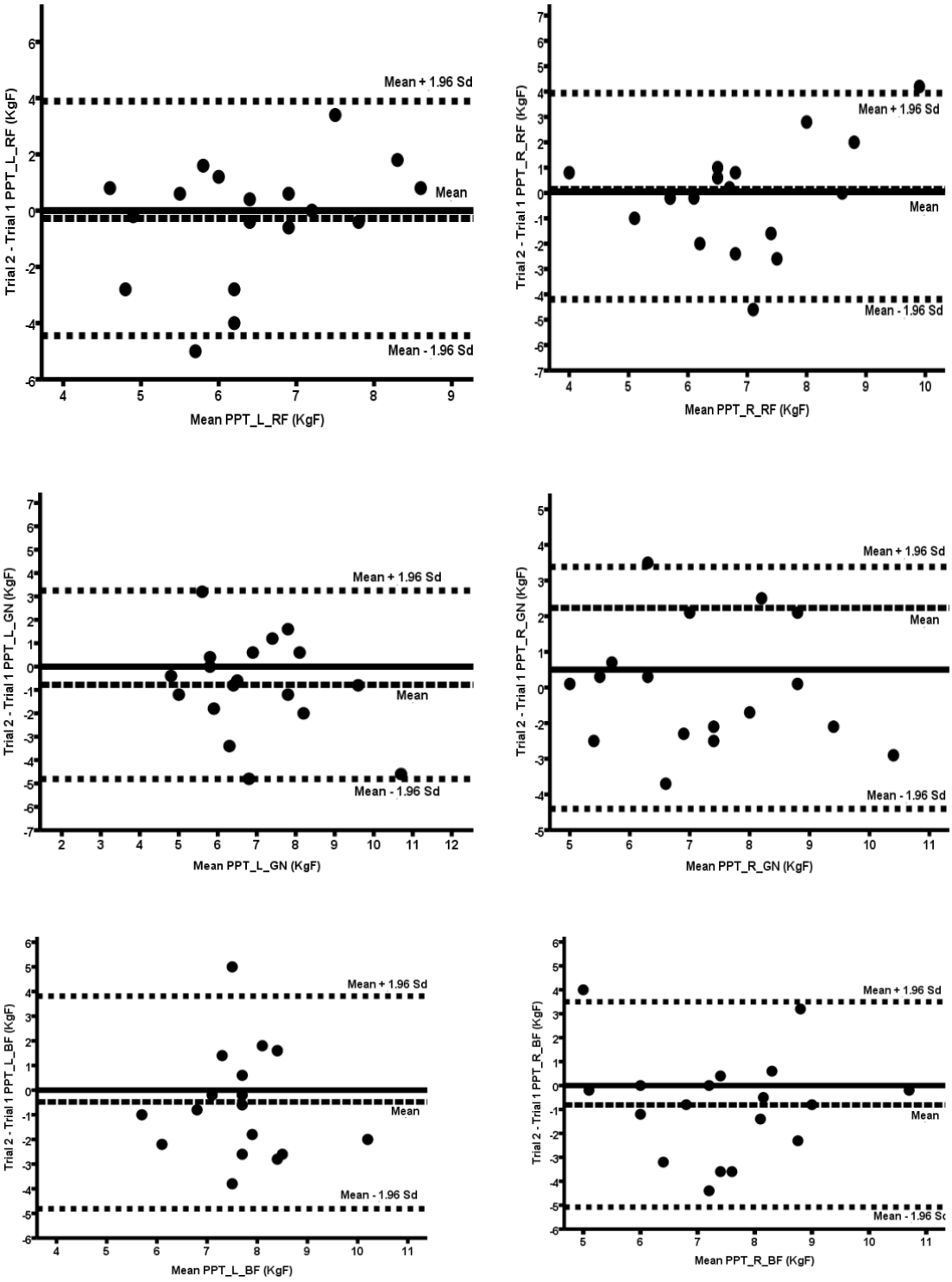

Figure 4.3 Bland-Altman plots showing difference between trial 1 and trial 2 in relation of the mean of the two trials for each individual for pain pressure threshold of the left rectus femoris, gastrocnemius and bicep femoris and right rectus femoris, gastrocnemius and bicep femoris. Dashed lines are plotted indicating the 95% limits of agreement using mean difference ± 1.96 x SD of difference

4.4 Discussion

The aim of study 1 was to establish the reproducibility of the indirect markers of EIMD across two separate trials. Trial 1 and trial 2 were separated by 4-7 days that are to be employed in Study 2.

The main findings of this study were that there was no systematic bias for any of the variables except CK ($p = 0.02$). Several measurement tools demonstrated good reliability, as the CV was < 10% (see Tables 4.5 – 4.8). However, several performance and functional measures; CK, flexibility, perceives soreness and PPT exhibited a CV of > 10% (see Table 4.5), suggesting low reproducibility. Additionally, CK, ROM of the right leg, VAS, limb circumference of the right gastrocnemius and left and right bicep femoris, and PPT (see tables 4.5 – 4.8), demonstrated questionable to low reliability (ICC < 0.7). However, the remaining measurement tools demonstrated good (ICC 0.7 – 0.8) to excellent reliability (ICC > 0.9) (Currell & Jeukendrup, 2006).

4.4.1 Reliability of indirect markers of muscle damage

There is an extensive debate in the literature concerning the reliability of CK as a marker of muscle damage (Baird et al., 2012). Values obtained for CK will reflect the relative amount of CK released, degree of enzyme activity of released CK, and the rate of clearance of CK from the blood (Thompson et al., 2006), which can be used as an indirect indicator of muscle damage (Manfredi et al., 1991, Baird et al., 2012). However, CK activity alone may not provide accurate reflections of structural damage to muscle cells (Magal et al., 2010) or the extent of muscle damage. There are several

factors that can influence the response of CK in individuals such as sex, mode of muscular actions, and duration of exercise (Clarkson & Ebbelling, 1988; Friden & Lieber, 2001; Newham, Jones & Clarkson, 1987). Thus, training state of participants and genetic predisposition (Newham, Jones & Clarkson, 1987) may affect the individuals CK response to the same exercise. CK was used in this study in order to make comparisons between other studies easier. There is a plethora of studies that use CK concentrations in the blood as a measure of muscle damage. Although this is seen as an unreliable measure, studies show a clear increase in CK activity after muscle damaging exercise (Bailey et al., 2007, Eston and Peters., 1999a, Clarkson et al., 1992, Davies et al., 2008, Davies et al., 2011a, Chen, 2003) with a gradual decrease in CK concentrations in the blood and a return to pre exercise levels.

Sit and reach tests are widely used as a measurement tool for evaluating hamstring flexibility (Ayala et al., 2012), as test with the greatest reliability and validity, and the least equipment and preparation is ideal (Hui and Yuen, 2000). Several studies have analysed the reliability of the sit and reach tests with ICC ranging from r = 0.91 to r = 0.99 (Davies et al., 2008, Ayala et al., 2012, Hui and Yuen, 2000, Hui et al., 1999, Lopez-Minarro et al., 2009, Perret et al., 2001). Using only the ICC as a reproducibility indicator these studies have subsequently reported high reliability. Reliability of the ROM measurements in exercise-induced muscle damage research has not been well documented (Watkins et al., 1991), and research has produced conflicting findings.

Jump mats are a valid measurement tool used to assess jumping test (Kenny et al., 2012), with squat jumps being the most reliable and valid tests for the estimation of dynamic strength of the lower limbs post muscle damaging exercise with a reported

coefficient of variance of 3.3% (Markovic et al., 2004). There are other jumping techniques used to assess strength, and there is a wide range of reliability reported (Ditroilo et al., 2011, Markovic et al., 2004, Moir et al., 2008, Slinde et al., 2008). This may be attributed to the differences in jump techniques used. Watkins et al., (1991) reported high to poor reliability of range of motion with ICC ranging from 0.59 – 0.89. Reliability between studies may be affected by different participant population demographics, time course, and equipment used and is therefore essential that researchers investigate the reproducibility of the measurement tools used (Atkinson & Nevill, 1998). Other factors that could affect the reliability are verbal encouragement, the use of music whilst exercising, feedback given to the subjects and the types of measurements taken (Currell & Jeukendrup, 2006) as this could influence the individuals overall performance.

Overall, PPT has demonstrated good to excellent reliability (Ylinen et al., 2007, Fischer, 1987), even though there are many different factors that can affect the reproducibility of PPT results. This good to excellent reliability demonstrated for pressure pain threshold is in conjunction with good reliability used for VAS, which is used to assess the perception of pain.

4.4.1.1 Creatine kinase

There was a significant difference in CK between trial 1 and 2 ($p = 0.02$, 95% CI = -0.10 to 0.36 IU/L). Additionally, the CV and ICC values for CK were 116% and 0.19 respectively, and therefore can be classified as displaying 'poor' reproducibility. Furthermore, the 95% LoA were -785.84, 1264.40 IU/L with a mean test-retest difference of 239.28 IU/L (see Figure 4.5). Conversely, previous studies have reported higher reproducibility values for CK (Newton et al., 2008, Chen et al., 2011). Furthermore, high intra-individual variability in CK activity in the present study may be attributed to individual differences and participants not adhering to pre test procedures between trial 1 and trial 2. Although the participants were instructed to refrain from physical activity, alcohol, and caffeine, and replicate the same diet, it is possible that this did not occur. The mean difference between trial 1 and trial 2 for CK activity was graphically shown with a Bland-Altman plot, in which the individual subject differences between trial 1 and trial 2 are plotted against the respective individual means (Atkinson & Nevill, 1998). This means that there is a rough indication of the systematic bias and random error by examining the direction and magnitude of the scatter around the zero line (Atkinson & Nevill, 1998), which demonstrates that upon retesting in trial 2, 95% of the CK activity fell in the 95% limits of agreement, with only one measurement falling outside of the 95% limits of agreement area, demonstrating an acceptable measurement error. Due to the systematic bias and large error associated with CK measure in combination with the magnitude of variation with regards to CK concentration at rest and following EIMD it is suggested that caution must be taken when using this measure to quantify the magnitude of damage, and assess the effectiveness of interventions (Currell & Jeukendrup, 2006).

4.4.1.2 Flexibility

In the present study, flexibility was assessed using the sit and reach test and demonstrated a CV of 17.4%, which can be classified as low reliability. However the ICC was 0.85, indicating good reliability (see Table 4.5). Differences in the reliability for the CV and ICC can be explained by the ICC's dependency on the sample heterogeneity (Hopkins, 2000). In contrast to the findings in the present study, several studies have explored the test-retest reproducibility of the sit and reach test (Davies et al., 2008, Hui and Yuen, 2000, Hui et al., 1999, Lopez-Minarro et al., 2009, Perret et al., 2001) with results demonstrating high reproducibility. Lopez-Minarro et al., (2009) demonstrated an ICC of 0.97, which shows higher reproducibility than the current study. This difference may be attributed to difference pelvic angles, which reflect with hamstring muscle extensibility, of the participants between the two studies (Cornbleet & Woolsey, 1996; Lopez-Minarro et al., 2009). Other factors that may influence the flexibility scores may be the spine and hip flexion (Lopez-Minarro et al., 2009), the forward reach, which is influenced by the contributions of the spine posture and anthropometric factors (such as height and weight). Furthermore, participants were able to see their scores when performing the sit and reach test in the present study which could increase the variability of measuring flexibility in the hamstrings due to motivation (Davies et al., 2008). The Bland-Altman graph for flexibility demonstrates that the two individual subject test re-test differences do not lie within the 95% limits of agreement and the difference between the two test lie within the 95% limits of agreement. The data is closely scattered around the zero line, indicating that there was no systematic bias or random error. The two measurements that fall outside the 95% limits of agree may be due to participants not adhering to pre-test measures.

4.4.1.3 Squat Jump

The CV (5.3%) and ICC (0.82) for squat jump in the present study demonstrated high reliability. The mean test-retest difference in SJ was 3.40 cm and the 95% LoA were -5.83, 6.79 cm. Participants were not informed of their jump scores in between individual jumps or between trial 1 and 2, and it is thought that this may have contributed to the high reproducibility, due to the elimination of motivation. In addition, standardised instructions and verbal encouragement were employed in order to reduce the variability between trials. Furthermore, participants were instructed to keep their hands on their hips or crossed over their chest during the jump, as it has been shown that arm swinging can increase the variability between jumps. Subsequently, the combination of these factors is thought to have contributed to the high reproducibility observed in the present study. In agreement with the findings in the present study previous research has shown CV ranging from 2.9% to 7.2% and ICC ranging from 0.75 to 0.97 (Ditroilo et al., 2011, Markovic et al., 2004, Moir et al., 2008, Slinde et al., 2008). However, in contrast to previous findings, Slinde et al., (2008) demonstrated higher ICC for jumps performed with arm swing 0.88, compared to 0.80 without arm swing as the arm swing represents a complex combination of arm - leg coordination (Markovic et al., 2004). Markovic et al., (2004) demonstrated an ICC of 0.93 for squat jump with arm swing, albeit good reliability, it can add to inconsistencies between different jump techniques. It has been suggested that caution needs to be taken when performing squat jumps with arm swing due to the inconsistencies with arm swing techniques, and therefore a no arm swing technique is preferred. The mean difference between trial 1 and trial 2 for squat jump was

graphically shown with a Bland-Altman plot. The Bland-Altman graph shows that upon retesting for trial 2 all but one subject fell within the 95% limits of agreement area. However, the measurements within the 95% limits of agreement were not evenly scattered around the zero line, which indicates that the data was not normally distributed. Overall, due to the high reproducibility of this measure in the present study, this measurement tool may be useful for researchers and practitioners to quantify any changes in neuromuscular function following a bout of EIMD. Measurements of squat jump are also easily replicated in the field, demonstrating justification for coaches and practitioners to use jump mats to quantify the effects of EIMD in a competitive, non-research based setting.

4.4.1.4 Range of motion

In the present study ROM showed high reliability with CV values of 4.8% and 5.6% for left and right leg respectively. Furthermore, the ICC demonstrated good to moderate reliability with values of 0.76 and 0.65 for left and right leg respectively. Watkins et al's (1991) demonstrated excellent reliability for goniometer knee flexion range of motion with ICC between 0.99 – 0.90. For ROM of both the left knee and the right knee there is an acceptable measurement error as the data lies within the 95% limits of agreement, with the exception of one measurement of the left leg. However, the measurements are scattered and do not fall close to or on the zero line. Overall, data for limb circumference fall within the 95% limits of agreement across the left and right rectus femoris, gastrocnemius and bicep femoris. However, all measurements have data that also falls outside the 95% limits of agreement. The left gastrocnemius data is close to the zero line, indicating good test retest reliability. The other

measurements demonstrate a wide scatter of data, indicating questionable re-test repeatability and wide variation within the same subject between trial 1 and trial 2 however, because these measurements are within 95% limits of agreement it is likely this method would not affect subsequent values. The differences in ROM reliability for this study and for other studies, may be due to the body position of the subject, test instructions and the time of day. All these factors will have an effect on reliability between studies.

4.4.1.5 Muscle Soreness

PPT showed poor reliability in the present study. PPT CV ranged from 16.5 – 23.2% and ICC ranged from 0.08 - 0.51 for left and right leg across all measurement sites (see Table 4.6 – 4.8 for further clarification). This is in contrast with Ylinen et al., (2007) who demonstrated ICC's varying from 0.78 to 0.93, which demonstrates good to excellent reliability. Variation in findings between Ylinen and colleagues results and findings in the present study may be attributed to significant differences of sensitivity between individual muscles. Ylinen's (2007) study looked at PPT of the neck, which includes muscles which are prone to be more sensitive than the muscle used for the current study. For example, the most sensitive muscles are in the upper body, where as the muscle situated in lower parts of the body are less sensitive and characterised by a high PPT (Fischer, 1987). ICC's also lack sensitivity to systematic changes in results, such as incremental improvements or deterioration due to repeated testing (Ylinen et al., 2007). Furthermore, there are many different factors that can influence the reproducibility of PPT, such as the difference in algometers used, the standardisation of pressure as well as when to express pain according to the

instructions given. In conjunction with PPT measurements using the algometer, for the current study VAS was also measured to determine the perception of pain at each time point. However, VAS demonstrated poor reliability (CV 61%, ICC 0.007) even though VAS is generally regarded as a valid and reliable measurement tool for acute pain measurements (Bijur et al., 2001). Despite this, the method of assessing VAS reliability has been criticised as providing an inflated estimate (Bijur et al., 2001). For VAS all measurements fall within 95% limits of agreement showing an acceptable measurement error, however the measurements are scattered and do not fall close to the zero line. However, the low reproducibility for VAS may to due to participants not following pre-test procedures correctly by participating in exercise either between trial one and trial two, or during study 2 data collection protocol. This may have subjected the participants to further damage after the downhill run.

4.4.2 Limitations and Implications

The sample size requirements for reproducibility studies vary within the literature. For example, one study has suggested that 50 study participants and at least three trials is ideal in order to determine adequate reliability (Hopkins, 2000). Furthermore, a minimum sample size of 20 has been recommended for reproducibility statistic (Atkinson and Nevill, 1998). The sample size in the present study was slightly less (n = 18). A smaller sample size can cause a large amount of inaccuracy when extrapolating to a population parameter (Hopkins, 2000), therefore, this needs to be considered in the present study.

4.4.3 Conclusion

The measurement tools used to assess the indirect markers of EIMD demonstrated varied levels of reproducibility. Measurements tools such as; CK, VAS, PPT and flexibility showed low test-retest reproducibility, whereas ROM and squat jump showed good reliability. Therefore, it could be suggested that several measurement tools of EIMD should be adopted in order to determine whether a true experimental change has occurred between time points. Due to differences in participant characteristics, experimental procedures, and statistical procedures employed, comparing different studies which assess reliability may be difficult. It is clear that measurements such as CK, VAS, PPT and flexibility does show low reliability or poor reproducibility, however in order to be able to compare this study with other studies these measurements will be used in study two. This will enables the chance to compare several studies which may not have used all the markers.

The current experimental chapter has answered the following research question: *To investigate the reproducibility and the reliability of the measurements tool used in this study to assess the signs and symptoms of EIMD.*

The measurements tools; CK, ROM, VAS, and pain pressure threshold all demonstrated low reproducibility. This demonstration of low reliability means that caution needs to be taken when interpreting the results found in study 2. However, even though range of motion and squat jump demonstrated good reliability, caution still needs to be taken when interpreting the results.

Chapter 5:

Experimental Study 2

The familiarisation (visit 1) and pre (visit 2) values obtained during data collection were reported in the previous experimental chapter on reproducibility. Only data from the pre (visit 2) and post trials (visits 3, 4 and 5) were used for statistical analysis in this experimental chapter.

5.1 Introduction

Exercise with a high eccentric component that is unaccustomed, typically leads to EIMD which can cause decrements in performance (Eston et al., 2003, Jakeman et al., 2010, Howatson & van Someren, 2008). These decrements may be due to several factors such as decreased neuromuscular function (Highton et al., 2009, Howatson, 2008), swelling and structural damage (Cleak and Eston, 1992, Eston et al., 2003) decrease in ROM (Eston and Peters, 1999, Clarkson and Newham, 1994), increase in limb circumference measurements (Friden et al., 1988), loss of contractile force (Friden et al., 1983, Newham et al., 1983), and sensation of soreness (Armstrong, 1984, Raastad et al., 2010). Therefore, a plethora of research has been conducted to investigate the most effective recovery strategies (Howatson and van Someren, 2008) to reduce the signs and symptoms of EIMD, and subsequently restore normal physiological processes, to enable an athlete to compete or train at an appropriate level (Halson, 2013). The choice of intervention employed will depend on the type of activity performed, the time until the next training session or event, and equipment and/or personnel available (Halson, 2013). The recovery strategies range from compression garments (Hill et al., 2013), nutritional strategies (Sousa et al., 2013), cryotherapy (Crystal et al., 2013, Ascensao et al., 2011, Bailey et al., 2007, Hausswirth et al., 2008, Bleakley et al., 2012) and water immersion (Brophy-Williams et al., 2011, Al Haddad et al., 2010, Barnett, 2006, Cochrane, 2004, Dixon et al., 2010, Halson et al., 2008, Howatson et al., 2008, Ingram et al., 2009, Jakeman et al., 2009, Minett et al., 2013).

Recently, the use of cold water immersion as a post-exercise recovery intervention has increased in popularity (Cochrane, 2004, Vaile et al., 2007). It is increasingly becoming a popular method of recovery due to the positive anecdotal benefits seen within the study (Halson et al., 2008). To date, literature supports the use of CWI to alleviate the signs and symptoms associated with EIMD (Eston & Peters, 1999, Newham et al., 1983; Yackzan et al., 1984; Newham and Jones, 1985; Friden et al., 1988; Nosaka et al., 1991; Cleak and Eston, 1992b; Howell et al., 1993; Nosaka and Clarkson, 1997). However, CWI may be more beneficial when compared to ice baths (Higgins, Heazlewood & Climstein 2011). Equally, the use of CWI may not be ecologically valid in the field, particularly in hot countries where controlling water temperature may be difficult, and subsequently other forms of water immersions may be more suitable. Furthermore, the use of CWT as a post exercise recovery strategy to attenuate EIMD has also become increasingly prevalent (Ingram et al., 2009, Cochrane, 2004, Coffey et al., 2004, Kuligowski et al., 1998, Vaile et al., 2007, Wilcock et al., 2006a) as CWT may be more tolerable for athletes with regards to temperature.

The proposed mechanisms responsible for the attenuation of the signs and symptoms associated with muscle-damaging exercise following CWT may be attributed to the vasodilation and vasoconstriction of the blood vessels, which occurs when alternating between hot water and cold water (Vaile et al., 2007), promoting the removal of waste products and the return of healthy regenerating cells. Furthermore, HWI may be more tolerable for individuals, and may be easier to control within the field or competition based setting if cold water is not available. HWI has been shown to increase tissue temperature (Kubo et al., 2005), increase local blood flow (Vaile et al., 2008), increase

muscle elasticity (Cochrane, 2004, Burke et al., 2001, Kubo et al., 2005), cause local vasodilation (Vaile et al., 2008), increase metabolic production (Cochrane, 2004) and reduce muscle spasm (Cochrane, 2004, Eston & Peters, 1999). Additionally, HWI decreases sympathetic nerve drive which causes vasodilation of local blood vessels and increase circulations which increases the supply of oxygen, antibodies and the ability to clear metabolites (Cochrane, 2004). However, if HWI causes an elevation in core temperature during subsequent prolonged exercise (particularly in hot environmental conditions), performance may be compromised (Ranalli et al., 2010) due to the increase in core temperature due to the immersion in hot water before exercise or activity. A recent study by Versey (2013) suggested that while HWI protocols appear unlikely to have a detrimental effect on performance, HWI is currently not recommended as a recovery technique. However, it is suggested that HWI may improve recovery of isometric squat force (Vaile et al, 2008b) with a 3.2% decrease in squat force for HWI compared to the control group (16% decrease) 72 h after muscle damaging exercise. Despite this, there is limited research of the effects of HWI alone on EIMD after eccentric exercise. Therefore, further research in this area is clearly warranted.

Subsequently, the aim of this chapter is to determine the effects of CWT and HWI on the signs and symptoms of EIMD damage after a single bout of downhill running. Downhill running was used in the study as the studies that employed DHR as a means of inducing muscle damage found DHR to be sufficient in producing muscle damage. The main findings of the previous experimental study (experimental study 1, chapter 4) found that there was no systematic bias for any of the variables except CK. This allows use of the other functional and performance measures as an adequate

measurement tool to quantify the effects of CWT and HWI on the signs and symptoms of EIMD and a bout of eccentric based exercise. However, caution needs to taken as although there was no systematic bias for all variables apart from CK, some functional and performance variables demonstrated low reproducibility

5.2 Methods

5.2.1 Participants

Eighteen healthy, physically active male participants (mean ± SD: height 177.8 ± 7.2 cm; body mass 74.3 ± 8.5 kg; age 21 ± 2 years) volunteered for this study. All participants completed necessary documentation and pre-test guidelines outlined in the general methodology (see section 3.1).

5.2.2 Experimental Design

Participants (n = 18) randomly allocated into three equal groups, visited the laboratory on five separate occasions. During visit 2, 3, 4 and 5 (separated by 24 hours) identical measures, in a specific order, were taken during each visit (1, 2, 3, 4 and 5, respectively). Additionally, all participants performed a downhill run during visit 2. Participants were randomly allocated into three equal groups (n = 6) of CWT, HWI and CON and immersed in water during visit 2, visit 3 and visit 4. The final visit (visit 5) at 72 h participants did not undergo water immersion. Participants were required to wear lose fitting shorts and a t-shirt, and refrain from changing their running shoes for each visit. Temperature and humidity in the laboratory ranged between 18 – 22^0C and

40 - 50%, respectively. Participants previously had their anthropometric measurements take during visit 1 (see section 3.1 in general methodology). See figure 5.1 for schematic of study 2.

5.2.3 Maximal Incremental Exercise

During visit 1, participants completed a treadmill maximal incremental exercise test ($\dot{V}O_{2max}$). Participants performed a 5 min warm-up at the starting speed of 6 $km \cdot h^{-1}$. Treadmill gradient was set at 1% to reflect the oxygen cost of running outdoors (Jones and Doust, 1996). After the warm up, the speed of the treadmill was increased to 7 $km \cdot h^{-1}$ and this marked the beginning of the test. Subsequently, speed increased by 1 $km \cdot h^{-1}$ every minute. Participants ran until volitional fatigue (Braun & Paulson, 2012). This test was used to determine maximal treadmill velocity ($\dot{V}O_{2max}$), which was used to determine their running speed for the downhill run. It has been suggested that taking physiological measurements such as expired gas or blood during a performance protocol will interfere with the performance of the subject (Currell & Jeukendrup, 2008). However, for this study gas analysis was monitored in order to determine when the participants reached their $\dot{V}O_{2max}$

5.2.4 Muscle Damaging Protocol

During visit 2 all participants completed a 40 minute downhill run at a -10% gradient and a velocity of 70% of maximum treadmill velocity as determined by the $\dot{V}O_{2max}$. Prior to beginning the run, participants warmed up for 5 min on a treadmill set at 0% gradient using self selected speed (Braun and Dutto, 2003). The average treadmill

velocity from the $\dot{V}O_{2max}$ were 16.8 ± 1.0 $km^{-1} \cdot h^{-1}$ and the average downhill run treadmill speed was 11.2 ± 2.4 $km \cdot h^{-1}$. Previous research utilising a similar protocol has consistently shown signs and symptoms associated with EIMD (Chen et al., 2007b, Braun and Dutto, 2003, Chen et al., 2008, Eston et al., 1996).

5.2.5 Water immersion

During visits 2 (20 min after DHR), 3 (24 h after DHR) and 4 (48 h after DHR) participants either received HWI, CWT or were placed in the control group. The time frame between muscle damaging exercise and water immersion chosen during visit 2 was employed as it represents a practical duration for recovery following exercise (Halson et al., 2008). Furthermore, the 20 min period allowed participants to change from their running clothes to minimal swimwear, and for those in the HWI or CWT groups, it enabled them time to insert a rectal thermometer in order to monitor core temperature during the water immersion.

5.2.6 Hot water immersion

During HWI, participants were immersed to the midsternal level with legs extended, in 38 ± 2° C (Vaile et al., 2008b, Kraft et al., 2011, Gill et al., 2006) in an inflatable bath (height; 42 cm, length; 220 cm x width; 60 cm and 180 L) for 40 min. Temperatures were continually monitored with a submersed water temperature gauge (Check Temp, Pocket Thermometer, Hanna Instruments, JPL) to ensure the water remained a constant temperature. If water became too cool, more hot water was added

to the bath. Plugs based at the bottom of the bath allowed easy removal of water, whilst adding more.

5.2.7 Contrast Water Therapy

Participants allocated to the CWT groups alternated between two baths of different temperatures. Cold water temperature was 11 ± 1 ℃. Although no specific temperature range has been determined for the cold water part of CWT, the sensation of pain begins at 15 ℃ (Wilcock et al., 2006a). Based on literature, hot water was 38 ± 2° C (Hamlin, 2007, Higgins et al., 2011, Robey et al., 2009). Participants alternated between each bath at 10 min intervals for a total of 40 min, commencing with hot and ending on cold (Cote et al., 1988). Participants were immersed to the midsternal level with legs extended.

5.2.8 Control

Participants were seated in an upright position with their legs extended for 40 min in the same position as if they were being immersed in the bath, however, for comfort reasons participants were sat on a plinth. Participants were allowed to wear lose fitting shorts, t-shirts and a jumper if required. The room was maintained at 22 ± 2℃ and 49 ± 3% relative humidity across all trials throughout the testing period.

Figure 5.1 Schematic of Study 2. Shaded areas represent time of data collection for the indirect markers of EIMD.

Visit	1	2			3		4		5
	4-7 days	Pre DHR	Post DHR	20 min	24 h	30 min	48 h	30 min	72 h
VAS				Water Immersion		Water Immersion		Water Immersion	
Anthropometrics									
$\dot{V}O_{2max/peak}$									
CK (IU/L)									
ROM (0)									
Tskin (℃)									
Flexibility (cm)									
SJ (cm)									
PPT (KgF)									
LC (cm)									

Timeline of the study; Pre DHR immediately before the downhill run, post DHR 2 minutes after the downhill run. Visit 2 water immersion was 20min after DHR, whilst visit 3 and 4 water immersion was 30 min after test measures; vVO_{2max} = vVO_{2max} to determine maximum treadmill velocity; CK = creatine kinase; ROM = range of motion; mm = millimetres; T_{skin} = skin temperature in degrees Celsius; cm = centimetre; SJ = squat jump; PPT = pressure pain threshold; LC = limb circumference; VAS = visual analogue scale; DHR = downhill run

5.2.9 Indirect Markers of EIMD Functional Measures

Indirect markers of EIMD obtained were limb circumference (rectus femoris, gastrocnemius, bicep femoris), PPT (rectus femoris, gastrocnemius, bicep femoris), CK, VAS, ROM, squat jump, and flexibility. Measurements were taken on every visit as outlined in the general methodology (see section 3.3)

5.3 Statistical Analysis

A between groups one-way analysis of variance (ANOVA) was performed for each functional and performance measure at visit 2 to ensure there was no significant difference between the control, hot water immersion and contrast water therapy groups at baseline. Quantile-quantile (Q – Q) plots were generated to infer the normality assumptions of the inferential tests were met. The instances where for normality assumptions were not met the relevant data were log log-transformed. Linear Mixed Models were used to determine the differences in variables (CK, squat jump, flexibility, ROM of the left and right knee, VAS, and PPT, limb circumference, and skin temperature of the left and right rectus femoris, gastrocnemius and bicep femoris) between the groups (CON, HWI and CWT) and across time (visit 2, 3, 4 and 5). In the incidence of a significant F statistic, Sidak adjusted Post Hoc tests were used to identify the differences between groups and time. 95% confidence intervals and effect sizes are presented. Significance was accepted as $p < 0.05$. Data is reported as the mean ± SD.

5.4 Results

Significant differences in baseline values between groups were found for squat jump ($F = 3.8$, $p = 0.04$), pressure pain threshold of the left rectus femoris ($F = 4.3$, $p = 0.03$) and VAS ($F = 5.2$, $p = 0.01$) (see Table 5.1). There were no other significant differences in the baselines values between groups for any other variable.

5.4.1 Creatine Kinase

A significant main effect was found for group ($F = 4.1$, $p = 0.04$). On average CON CK activity was 63% higher when compared to CWT CK activity ($p = 0.04$, 95% CI: 10.6, 915.23 UI/L). A significant main effect was found for time ($F = 5.2$, $p = 0.001$). On average CK activity was 72% higher at 24 h post muscle damaging exercise than pre exercise ($p = 0.001$, 95% CI: 188.3, 948.1 UI/L). CK activity was 55% lower at 72 h post DHR compared to 24 h post DHR ($p = 0.02$, 95% CI: -8.14.5, -54.7 UI/L). No significant interaction effect was found ($F = 1.09$, $p = 0.39$).

5.4.2 Squat Jump

There was a significant main effect for group ($F = 8.414$, $p = 0.004$) in the HWI group. Overall mean squat jump values were 27% higher in the HWI group compared to the CON group ($p = 0.008$, 95% CI: 20.6, 17.8 cm) and overall mean squat jump values in the HWI group were on average 26% higher than the CWT group ($p = 0.010$, 95% CI: 2.3, 17.5 cm). Additionally, there was a significant main effect for time ($F = 5.2$, $p = 0.003$). At 24 h post muscle damaging exercise, mean squat jump values were 14%

lower than pre exercise (p = 0.009, 95% CI: -10.3, -1.1 cm). At 48 h post exercise mean squat jump values were on average 11% lower than pre muscle damaging exercise (p = 0.035, 95% CI: -9.5, -.2 cm). No significant interaction effect was found (F = 1.8, p = 0.123).

5.4.3 Flexibility

No significant main effect for group was found (F = 0.2, p = 0.854). A significant main effect for time was found (F = 0.4, p = 0.009). At 24 h post muscle damaging exercise mean flexibility scores were on average 17% lower than pre exercise (p = 0.018, 95% CI: -5.6, -.4 cm). At 72 h post muscle damaging exercise mean flexibility scores were on average 14% higher than 24 h post exercise (p = 0.027, 95% CI: .2, 5.4 cm). No significant interaction effect was found (F = 1.2, p = 0.317).

5.4.4 Limb Circumference

No significant main effect for group was found for left rectus femoris, left gastrocnemius, and bicep femoris (F = 1.8, p = 0.194, F = 0.6, p = 0.570, and F = 1.2, p = 0.326, respectively). No significant main effect for time was found for left rectus femoris, left gastrocnemius, and bicep femoris (F = 1.5, p = 0.224, F = 1.1 p = 0.354, and F = 2.3, p = 0.084, respectively). No significant interaction effect was found for left rectus femoris, left gastrocnemius, and left bicep femoris (F = 1.8, p = 0.114, F = 1.0, p = 0.429, and F = 2.3, p = 0.051, respectively).

No significant main effect for group was found for right rectus femoris, gastrocnemius, and bicep femoris (F = 2.0, p = 0.161, F = 0.9, p = 0.416, and F = 2.0, p = 0.159, respectively). No significant main effect for time was found for right rectus femoris, gastrocnemius, and bicep femoris (F = 0.2. p = 0.892, F = 0.3, p = 0.803, and F = 1.1, p = 0.353, respectively). A significant interaction effect was found for right rectus femoris (F = 2.7, p = 0.026) at 48h post muscle damaging exercise. Post hoc test reveal that on average the mean values in the CWT group were 10.2% lower than the HWI group (p = 0.025, 95% CI: -11.2, -.6 mm) at 48 h post muscle damaging exercise. No significant interaction effect was found for right gastrocnemius and bicep femoris (F = 1.6, p = 0.178 and F = 0.8, p = 0.594 respectively).

5.4.5 Skin Temperature

No significant main effect for group was found for left rectus femoris, gastrocnemius, and bicep femoris (F = 1.5, p = 0.249, F = 1.7, p = 0.208, and F = 0.8, p = 0.483, respectively). A significant main effect for time was found (F = 3.8, p = 0.017). For left rectus femoris there was on average a 4.4% increase in skin temperature from pre muscle damaging exercise to 48 h post exercise. A trend was found for the interaction effect (F = 2.3, p = 0.053) and Post hoc tests reveal a significant effect at Pre. On average skin temperature at Pre was 9.1% higher in the HWI group, compared to the CWT group (p = 0.027, 95% CI: .2, 4.8 ^{0}C). A significant effect was found for the CWT group. On average skin temperature at Pre was 6.4% lower than 24h post exercise (p = 0.043, 95% CI: -3.8, -.03 ^{0}C), 10.1% lower than 48 h post exercise (p < .01, 95% CI: -5, -1.3 ^{0}C) and 7.5% lower than 72 h post exercise (p = 0.030, 95% CI: -4.4, -.2 ^{0}C). No significant main effects for time were found for left gastrocnemius

and bicep femoris (F = 0.03, p = 0.992 and F = 8.40, p = 0.479, respectively). No significant interaction was found for left gastrocnemius and bicep femoris (F = 0897, p = 0.506 and F = 1.174, p = 0.338, respectively).

No significant main effect for group was found for right rectus femoris, gastrocnemius, and bicep femoris (F = 0.3, p = 0.727, F = 1.6, p = 0.215, and F = 0.6, p = 0.539, respectively) and no significant main effect for time was found (F = 0.5, p = 0.692, F = 2.7, p = 0.102, and F = 0.1, p = 0.932, respectively). No significant interaction effect was found for right rectus femoris, gastrocnemius, and bicep femoris (F =0 .4, p = 0.858, F = 1.8, p = 0.120, and F = 1.2, p = 0.311, respectively.

5.3.6 Muscle Soreness; Pressure Pain Threshold and VAS

5.3.6.1 VAS

No significant main effect for group was found for VAS (F = 2.9, p = 0.086). A significant main effect for time was found (F = 27.5, p = 0.000). On average at 24 h post muscle damaging exercise VAS scores were 59.9% higher than Pre (p = 0.000, 95% CI: .6, 2.5), 86.6% higher than 48 h post exercise (p 0.000, 95% CI: 1.2, 2.9) and 225.1% higher than 72 h post exercise (p = 0.000, 95% CI: 2.1, 3.9). No significant interaction was found (F = 1.03, p = 0.413).

5.3.6.2 Pressure Pain Threshold

5.3.6.1 Left Rectus Femoris

No significant main effect for group was found (F = 2.2, p = 0.145). A significant main effect for time was found (F = 6.9, p = 0.001). At 24 h post exercise there was a 19.2% decrease in mean values compared to pre exercise (p = 0.020, 95% CI: -2.2, -.1 KgF). At 72 h post exercise there was a 33.5% increase in mean values from 24 h post exercise (p < .01, 95% CI: .6, 2.9 KgF). No significant interaction effect was found for group x time (F = 0.2, p = 0.594).

5.3.6.2 Left Gastrocnemius

No significant main effect for group was found (F = 2.8, p = 0.085). No significant main effect for time was found (F = 2.4, p = 0.080). No significant interaction effect was found (F = 1.1, p = 0.393).

5.3.6.3 Left Bicep Femoris

No significant main effect for group was found (F = 1.8, p = 0.207). A significant main effect was found for time (F = 7.1, p = 0.001). There was a 29.5% increase in mean values from 24 h post exercise to 72 h post exercise (p = 0.003, 95% CI: .6, 3.7 KgF). There was a 31% increase in mean values from 48 h post exercise to 72 h post exercise (p = 0.003, 95% CI: .7, 3.7 KgF). A significant interaction effect for group x time was found (F = 3.4, p = 0.007). Pairwise comparisons reveal a significant interaction effect at 72 h post muscle damaging exercise. On average values in the CWT group are 36% higher than the CON group (p = 0.025, 95% CI: .3, 5.7 KgF) and 35% higher than the HWI group (p = 0.027, 95% CI: .3, 5.7 KgF). Post Hoc tests reveal that in the CON

group, values at 48 h post exercise were on average 35.7% lower than values at pre exercise (p = 0.007, 95% CI: -5.7, -.7 KgF). In the CWT group, values at 72 h post exercise were 52.5% higher than pre exercise values (p = 0.001, 95% CI: 1.3, 6.4 KgF) and 47.9% higher than 48h post exercise (p = 0.002, 95% CI: 1.1, 6.2 KgF).

5.3.6.4 Right Rectus Femoris

A significant main effect for group was found (F = 5.7, p = 0.014). On average values in the CWT group were 25.9% higher than the CON group (p = 0.020, 95% CI: .3, 3.5 KgF). No significant main effect for time was found (F = 2.4, p = 0.084). No interaction effect for group x time was found (F = 1.0, p = 0.436).

5.3.6.5 Right Gastrocnemius

No significant main effect for group was found (F = 1.6, p = 0.236). A significant main effect for time was found (F = 6.8, p = 0.001). On average, values at 72 h post exercise were 19.5% higher than pre exercise values (p = 0.018, 95% CI: .19, 2.8 KgF), 30.8% higher than 24 h post exercise (p = 0.001, 95% CI: .8, 3.4 KgF) and 16.9% higher than 48 h post exercise (p = 0.45, 95% CI: .01, 2.6 KgF). No significant interaction effect was found for group x time (F = 1.2, p = 0.320).

5.3.6.6 Right Bicep Femoris

No significant main effect was found for group (F = .2, p = .727). A significant main effect for time was found (F = 5.1, p = 0.004). On average values at 72 h post exercise

were 29.9% higher than 24 h post exercise (p = 0.002, 95% CI: .6, 3.5 KgF). No significant interaction effect for group x time was found (F = 1.8, p = 0.131).

5.3.7 Range of Motion

No significant main effect for group for left (F = 1.2, p = 0.343) and right (F = 2.8, p = 0.160) ROM was found. No significant main effect for time was found for right ROM (F = 1.3, p = 0.274). However, a significant main effect for time was found (F = 4.6, p = 0.007) for left ROM. There was a 5.8% decrease in ROM from 24 h post exercise to 72 h post exercise (p = 0.006, 95% CI: -6.4, $-.8^0$). No significant interaction effect was found for group x time for the left and right knee ROM (F = 1.8, p = 0.131, F = 0.7, p = 0.682, respectively).

Table 5.1 Values obtained for CK, squat jump, flexibility, ROM of the left and right leg (^{0}) and VAS, limb circumference (cm), pressure pain threshold (KgF) and skin temperature (^{0}C) of the left and right leg for rectus femoris, gastrocnemius and bicep femoris for Pre (baseline). Raw data was reported for CK.

	CON		HWI		CWT			
	Mean±SD	95% CI	Mean±SD	95% CI	Mean±SD	95% CI	F ratio	p value
CK (IU/L)	2.3±0.3	1.9-2.6	2.3±0.2	2.0-2.5	2.3±0.1	2.1-2.4	0.09	0.91
SJ (cm)	1.6±0.6	1.6-1.7	1.6±0.0	1.6-1.7	1.6±0.0	1.6-1.7	3.8	0.05
Flexibility (cm)	19.1±2.9	16.0-22.1	17.4±9.7	7.2-27.6	14.6±9.2	4.9-24.3	0.49	0.61
ROM L (^{0})	61.2±3.1	57.9-64.5	59.4±3.7	55.4-63.3	58.2±10.4	47.2-69.1	0.32	0.73
ROM R (^{0})	62.8±4.9	57.5-68.0	56.6±4.4	52.0-61.2	55.5±7.2	47.9-63.1	2.83	0.09
VAS	3.3±0.4	2.2-4.4	1.67±0.8	0.8-2.5	1.2±0.4	2.0-4.6	5.21	0.02*
LC_L_RF	1.9±0.0	1.6-1.7	1.7±0.03	1.6-1.7	1.6±0.02	1.6-1.6	2.84	0.89
LC _L_GN	1.7±0.0	1.6- 1.7	1.7±0.03	1.6-1.7	1.7±0.03	1.7-1.7	1.11	0.26
LC _L_BF	1.5±0.3	1.5-1.6	1.6±0.06	1.54-1.6	1.5±0.04	1.5-1.6	1.64	0.23
LC _R_RF	1.7±0.0	1.7-1.7	1.7±0.02	1.7-1.7	1.7±0.02	1.7-1.7	1.15	0.34
LC _R_GN	1.5±0.0	1.5-1.6	1.5±0.02	1.5-1.6	1.5±0.03	1.5-1.5	1.15	0.34
LC _R_RF	1.7±0.03	1.7-1.7	1.7±0.02	1.7-1.7	1.7±0.02	1.7-1.7	1.15	0.34
LC _R_GN	1.5±0.03	1.5-1.6	1.5±0.02	1.5-1.6	1.5±0.03	1.5-1.5	1.15	0.34
LC _R_BF	1.6±0.05	1.6-1.7	1.6±0.02	1.6-1.7	1.6±0.02	1.6-1.6	1.43	0.27

Table 5.1 continued

	CON		HWI		CWT			
	Mean±SD	95% CI	Mean±SD	95% CI	Mean±SD	95% CI	F value	p value
PPT_L_RF	6.8±1.2	5.4-8.1	5.5±0.9	4.6-6.5	7.3±1.0	6.2-8.4	4.23	0.03*
PPT_L_GN	8±2.5	5.2-10.7	6.1±0.7	5.3-6.9	7.9±2.2	5.6-10.2	1.65	0.26
PPT_L_BF	8.9±1.6	7.2-10.7	7.5±0.9	6.5-8.4	7.3±2.0	5.8-8.8	2.51	0.12
PPT_R_RF	6.7±1.0	5.5-7.8	8.1±2.4	4.5-8.3	8.2±1.0	6.2-8.9	1.10	0.40
PPT_R_GN	6.4±1.8	5.5-10.7	7.0±1.4	5.47-8.	7.6±1.6	6.0-9.0	0.53	0.60
PPT_R_BF	7.6±1.3	7.1-9.3	7.5±1.4	5.91-9.2	7.8±1.8	4.6-10.6	0.21	0.82
Tskin_L_RF	29.7±0.8	28.8-30.7	30.4±1.6	28.7-32.1	27.9±2.9	24.8-31.0	2.51	0.12
Tskin _L_GN	29±1.1	27.8-30.2	30.5±0.6	29.9-31.2	28.9±1.8	26.9-30.8	3.07	0.78
Tskin _L_BF	30.9±1.3	29.5-32.3	31.2±1.0	30.1-32.3	29.1±2.1	26.8-31.3	3.33	0.06
Tskin _R_RF	30.2±1.6	28.5-31.8	30.6±0.5	30.0-31.1	29.9±1.5	28.2-31.5	0.45	0.65
Tskin _R_GN	29±0.7	28.4-29.8	30±0.4	29.5-30.5	32.5±4.5	27.8-37.3	2.65	0.10
Tskin _R_BF	30.4±2.0	28.2-32.6	30.6±0.2	30.3-30.9	29.9±1.3	28.6-31.3	0.34	0.72

CON = control group; HWI = hot water immersion; CWT = contrast water therapy; 95% CI = 95% confidence intervals; Mean±SD = mean and standard deviation; CK = creatine kinase; SJ = squat jump; ROM L = range of motion of the left leg; ROM R; range of motion of the right leg; VAS = visual analogue scale; LC = limb circumference; PPT = pain pressure threshold; T_{skin} = skin temperature; RF = rectus femoris; GN = gastrocnemius; BF = bicep femoris; Units cm = centimetre; mm = millimetre; KgF = kilogram of force; ℃ = degrees Celsius; * = significant at $p < 0.05$.

Table 5.2 Values obtained for CK, squat jump, flexibility, ROM of the left and right leg, VAS for Pre. 24, 48 and 72 h for the CON, HWI and CWT

Condition		Pre	24h	48h	72h
		Mean±SD	Mean	Mean	Mean
CK (IU/L)	CON	237.2±226.0	1314.3±1181.0	682.3±400.9	600.3±421.5
	HWI	225.8±148.1	667.2±165.4	402±143.1	352.8±94.8
	CWT	206.5±58.3	425.1±265.0	330.±3248.4	160.8±59.4
SJ (cm)	CON	42.2±5.9	37.7±4.9	33.8±6.	38.7±7.9
	HWI	49.2±4.1	47.2±4.5	48.0±5.7	48.9±4.5
	CWT	42.6±4.4	31.8±13.7	37.5±5.8	41.9±4.7
Flexibility (cm)	CON	18.5±2.9	16±5.2	14.7±6.5	14.7±6.5
	HWI	16.3±9.8	13.6±68.9	16.7±11.0	17.0±11.1
	CWT	16.3±8.3	12.6±9.0	14.8±9.2	15±9.2
ROM L (^{0})	CON	61.2±3.1	64.8±4.9	64.6±3.1	62.1±6.4
	HWI	59.4±3.7	61.2±4.4	60.0±4.6	59.5±6.3
	CWT	58.2±10.4	60.4±9.3	57.6±11.1	54.2±8.
ROM R (^{0})	CON	62.8±4.9	63.1±7.0	63.3±±7.1	60.8±5.4
	HWI	56.6±4.4	59.4±6.2	68.3±23.2	57.0±6.
	CWT	55.53±7.26	56.8±4.5	57.8±6.4	56.5±6.6
VAS	CON	3.3±1.0	4.3±1.5	2.1±1.3	1.3±0.8
	HWI	1.6±0.8	3.8±0.4	1.8±0.7	1.3±0.5
	CWT	1.2±0.4	4.8±0.9	2.6±1.9	1.3±0.8

See table 5.5 for abbreviations

Table 5.3 Values obtained for limb circumference (cm), pressure pain threshold (KgF), skin temperature (^{0}C) of rectus femoris, gastrocnemius and bicep femoris of the right leg

Condition		Pre	24h	48h	72h
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
	LC_RF	54.6±4.0	57.1±2.5	56.16±1.94	56.33±2.87
CON	LC_GN	38.4±2.8	38.3±2.2	38.58±2.57	37.75±2.48
	LC_BF	49.3±6.2	49.6±3.6	45.16±7.02	48.91±3.32
	LC_RF	56.8±3.2	55.5±4.9	57.83±3.48	56.66±3.07
HWI	LC_GN	38.0±1.7	38.0±1.8	37.83±2.04	37.83±1.57
	LC_BF	48.7±2.4	49.3±2.9	48.50±3.72	48.50±3.20
	LC_RF	53.91±3.1	53.1±3.1	51.91±4.94	53.75±3.79
CWT	LC_GN	36.5±2.1	36.3±1.6	35.91±2.76	38.00±3.80
	LC_BF	45.7±1.9	45.5±3.3	45.41±3.35	45.83±3.12
	PPT_RF	6.7±1.8	5.9±2.4	5.53±2.26	6.53±1.07
CON	PPT_GN	8.1±2.4	7.0±2.5	7.13±2.13	9.36±2.04
	PPT_BF	8.2±1.0	7.6±1.9	6.61±1.41	9.50±1.11
	PPT_RF	6.4±1.8	5.2±1.2	5.43±1.41	6.50±1.11
HWT	PPT_GN	7.0±1.4	5.7±0.9	6.80±0.63	7.75±2.07
	PPT_BF	7.6±1.6	6.1±1.6	7.83±2.43	8.23±1.59

See table 5.5 for abbreviations

Table 5.4 Values obtained for pressure pain threshold (KgF), skin temperature (^{0}C) of rectus femoris, gastrocnemius and bicep femoris of the right leg

Condition		Pre	24h	48h	72h
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
	PPT_RF	7.2±0.9	6.3±1.8	7.9±2.0	8.9±0.9
CWT	PPT_GN	6.8±1.2	7.3±3.3	7.9±1.9	9.7±3.4
	PPT_BF	7.3±3.5	6.3±1.4	8.0±2.8	8.4±0.5
	Tskin_RF	30.2±1.6	29.5±1.3	29.5±1.2	30.9±4.4
CON	Tskin_GN	29.1±0.7	29.4±0.6	29.0±0.4	29.5±0.6
	Tskin_BF	30.4±2.0	30.0±1.7	29.5±1.6	29.5±1.1
	Tskin_RF	30.6±0.5	30.7±1.1	30.7±1.3	30.8±0.8
HWI	Tskin_GN	30.1±0.4	29.9±0.7	30.0±1.1	30.6±0.7
	Tskin_BF	30.5±0.1	30.3±1.3	30.5±1.2	31.2±1.0
	Tskin_RF	29.1±1.4	29.9±1.2	30.6±1.1	30.3±1.7
CWT	Tskin_GN	34.0±5.0	29.4±1.8	29.6±1.6	29.6±1.2
	Tskin_BF	30.0±1.64	31.0±2.7	31.4±3.62	31.1±1.9

See table 5.5 for abbreviations

Table 5.4 continued Values obtain for limb circumference, pressure pain threshold, skin temperature of rectus femoris, gastrocnemius and bicep femoris of the left leg

Condition		Pre	24h	48h	72h
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
	LC_RF	49.6±4.2	49.1±2.4	47.8±3.1	47.6±3.0
CON	LC_GN	55.2±3.9	55.9±2.9	56.5±2.8	57.5±3.7
	LC_BF	38.5±2.4	38.6±3.6	38±2.3	38.0±2.3
	LC_RF	49.6±4.2	49.1±2.4	47.8±3.1	47.6±3.0
HWI	LC_GN	56.4±3.7	57.2±3.3	57.5±3.2	56.2±3.8
	LC_BF	41.1±6.8	37.6±1.8	37.6±1.9	37.5±1.8
	LC_RF	46.3±2.7	47±2.7	47.5±2.9	46.7±3.9
CWT	LC_GN	53.3±3.2	53.8±3.4	53.5±3.9	53.0±3.6
	LC_BF	36.8±2.4	39.3±6.9	36.5±1.9	36.2±2.1
	PPT_RF	6.8±1.2	5.3±1.3	5.5±1.1	6.8±1.7
CON	PPT_GN	8±2.5	6.9±1.7	6.3±1.6	8.8±1.7
	PPT_BF	8.9±1.6	6.9±1.3	5.7±0.4	8.2±1.1
	PPT_RF	5.5±0.9	5.2±1.1	5.6±1.2	6.3±1.0
HWT	PPT_GN	6.1±0.7	5.3±1.0	6.5±0.9	6.6±1.2
	PPT_BF	7.5±0.9	6.3±1.3	7.7±1.7	8.2±1.6

See table 5.5 for abbreviations

Table 5.5 Values obtain for pressure pain threshold and skin temperature of rectus femoris, gastrocnemius and bicep femoris of the left leg

CWT	PPT_RF	7.16±1.05	5.24±0.59	6.56±2.98	8.14±2.07
	PPT_GN	7.90±2.68	8.05±3.76	6.90±1.94	8.60±4.12
	PPT_BF	6.75±1.18	8.20±3.66	7.05±2.94	11.25±3.05
CON	Tskin_RF	29.78±0.88	29.76±0.82	30.06±1.30	29.35±1.55
	Tskin_GN	29.05±1.16	29.35±2.11	28.90±1.20	28.96±1.41
	Tskin_BF	30.93±1.32	30.81±1.67	30.68±1.59	29.86±1.71
HWI	Tskin_RF	30.36±1.79	30.68±1.73	30.90±1.43	31.12±0.48
	Tskin_GN	30.54±0.71	29.86±1.81	30.20±1.20	30.46±0.70
	Tskin_BF	31.18±1.11	30.44±2.29	30.94±1.24	30.96±1.36
CWT	Tskin_RF	26.95±3.27	29.10±1.86	30.82±1.59	29.75±1.88
	Tskin_GN	29.10±2.09	29.42±1.92	29.62±2.16	29.17±1.48
	Tskin_BF	29.15±2.40	30.87±2.62	30.05±3.33	30.85±2.15

CON = control group; HWI = hot water immersion; CWT = contrast water therapy; 95% CI = 95% confidence intervals; Mean±SD = mean and standard deviation; CK = creatine kinase; SJ = squat jump; ROM L = range of motion of the left leg; ROM R; range of motion of the right leg; VAS = visual analogue scale; LC = limb circumference; PPT = pain pressure threshold; T_{skin} = skin temperature; RF = rectus femoris; GN = gastrocnemius; BF = bicep femoris; cm = centimetre; mm = millimetre; KgF = kilogram of force; ℃ = degrees Celsius.

5.4.1 Discussion Overview

The purpose of this investigation was to examine the effects of contrast water therapy and hot water immersion on the signs and symptoms of exercise-induced muscle damage after a single bout of downhill running. The main findings of the study found that the downhill run was successful in producing a sufficient amount of muscle damage as all measurement tools peaked at 24 h post muscle damaging exercise. For example, there was an overall increase in CK activity and VAS, and an overall decrease in squat jump, flexibility, PPT and ROM of the left knee at 24 h after the downhill run. Overall there were peak decrements at 24 h for CK, PPT, flexibility, squat jump, and VAS in the CWT and HWI groups whilst the peak decrements in the CON for PPT, flexibility and squat jump at 48 h post downhill run.

5.4.2 Creatine Kinase

Overall CK activity in the CON group were 173% higher than CK activity in the CWT group and 88% higher than CK activity in the HWI group, suggesting that water immersion may attenuate CK activity in the blood. Additionally, overall mean CK activity were 251% higher at 24 h post exercise compare to pre-exercise and remained elevated at 72 h (59%) following the DHR. This shows that the muscle damage protocol was successful in increasing CK activity in the blood. Furthermore, the peak in CK 24 h post DHR agrees with previous research that have reported a peak in CK 12-24 h post muscle damaging exercise with increases ranging from 100 to 600 UI (Clarkson & Hubal, 2002). Additionally, similar to this study, Chen et al (2009) demonstrated a 231% increase in CK activity 24 h post DHR (-16% gradient for 30min

at 70% $\dot{V}O_{2max}$. However, in contrast to the findings in the present study Chen and colleagues observed their peak in CK 48 h post DHR (Chen et al., 2009). Differences in findings may be attributed to the different downhill running protocol used to induce muscle damage. Along with the downhill running, another factor that may affect the findings is the body fat percentage of the participants used in the study. Body fat has the potential to influence the magnitude of damage and the effectiveness of the intervention that is implemented (Hickner et al., 2001). In support of findings in the present study Eston and colleagues reported a 267% increase in CK activity 24 h post downhill running (Eston et al., 1996), with CK activity only returning to baseline on day 7 (see Table 2.1, Chapter 2 for further clarification). Conversely, after high force eccentric exercise (e.g. elbow extension on an isokinetic dynamometer), CK does not peak until approximately 4 to 6 days post exercise with CK values increasing to over 2,000 UI (Nosaka & Clarkson, 1991, Clarkson & Hubal, 2002). However, studies have also found that CK peaks earlier than 4 – 6 days (Clarkson & Hubal, 2002, Clarkson & Nosaka, 1992, Newham et al., 1983).

In the CON and HWI group CK activity did not return to pre exercise levels 72 h post DHR. However, CK activity did return to baseline in the CWT group. There is general consensus within the literature that the body is capable of clearing released muscle components such as CK back to baseline levels within 7 - 9 days (Sayers and Clarkson, 2003, Totsuka et al., 2002). However, with CWT (i.e. a combination of hot and cold water immersion), CK activity may be cleared from the blood stream quicker. This increase in clearance rate may be attributed to the vasoconstriction and vasodilation of the blood vessels that occurs during contrast water therapy. Even though CWI was not conducted in this study, it is important to determine the effects of CWI in

attenuating CK activity as this may explain the mechanisms of CWT due to CWI being a popular method of recovery using water immersion. Following muscle damaging exercise Eston & Peters (1999) reported an 85% increase in CK from baseline in a control group 48 h after eccentric elbow flexion exercise, compared to a 24% increase from baseline to 48 h post exercise in the a CWI group. These findings demonstrate that CWI can either increase the removal of CK from the blood and/or attenuate the release of CK from the muscle cell (Eston and Peters, 1999). However, it is presently unclear as to whether this increase in removal of CK and/or decrease in release of CK is due to water temperature or the effects of hydrostatic pressure (Wilcock et al., 2006). Alternatively, Bailey et al., (2007) reported no significant difference in CK concentration following CWI compared to a control group. The differences in findings between these studies may be due to Eston and Peters (1999) implementing an upper body muscle damaging protocol, and Bailey and colleagues utilised a lower body muscle damaging protocol (Eston and Peters, 1999, Bailey et al., 2007). Similarly, Sellwood et al., (2007) showed no significant difference in CK activity between the control group and CWI group. The lack of treatment effect may be due to the muscle damage protocol only inducing low levels of muscle damage and the short duration of water immersion (3 x 1 min).

In summary, it is apparent that the addition of HWI and CWT may assist in either decreasing the amount of CK released into the blood or attenuate the removal of CK in the blood. Although, CK is an indicator of muscle damage, an attenuated increase and/or increase removal rate of CK does not contribute to the improvement of subsequent performances or the increase rate in recovery after muscle damaging exercise (Baird et al., 2012). Furthermore, CK activity may not provide a full and

accurate representation of the structural damage to the muscle after damaging exercise (Magal et al., 2010) or the magnitude of muscle damage (Friden & Lieber, 2001), and may not correlate well with other indirect markers of muscle damage (Nosaka et al., 2002). As demonstrated in study 1, the reproducibility of CK as a marker of muscle damage is low, which further adds to the unreliability of CK as a marker of muscle damage.

5.4.3 Squat Jump

In the current study squat jump height was on average higher in the HWI group compared to the CON group (26%) and the CWT group (25%) overall. This suggests that there was less of a decrease in squat jump height post muscle damaging exercise with the addition of hot water as a recovery intervention. Furthermore, 24 h following the DHR, squat jump height decreased by 11% in the CON group, 26% in the CWT, and 4% in the HWT group. However, there was a significant difference ($p = 0.004$) between groups at baseline; squat jump height in the HWI group was on average 16% higher than the squat jump height in the CON group and CWT group, and therefore, caution should be taken when interpreting these findings, as any changes in squat jump height could be due to the fact that the groups were different to begin with as opposed to the intervention and treatment. Additionally, there was no interaction effect in this study, suggesting that HWI and CWT did not attenuate the decrement in neuromuscular function compared to CON. Conversely, Vaile et al (2008), found CWI significantly attenuated decrements in squat jump height 24 h (7.15% decrease), 48 h (3.49% decrease) and 72 h (0.02% decrease) after a simulated netball match circuit when compared to a control group in regionally trained female netball players. In

agreement with the results in the present study Vaile et al., (2008) demonstrated no significant difference in jump height when individuals were exposed to HWI following EIMD. Therefore, in addition to hydrostatic pressure, water temperature may also play a role in attenuating neuromuscular dysfunction following damaging exercise.

5.2.4 Flexibility

In the current study, there was no significant main effect for group. However, mean flexibility was on average 17% lower at 24 h post DHR compared to Pre DHR. This means that the muscle damaging protocol was successful in causing decrements in flexibility due to the increase in muscle stiffness brought on by EIMD. Nevertheless, no significant interaction effect was found, suggesting that HWI and CWT does not attenuate the decrement in flexibility compared to a CON group following a 40 min DHR. In contrast to the findings in the present study, a significant difference in flexibility following EIMD has been found when individuals are exposed to CWI and CWT, compared to a control group 48 h and 72 h following a simulated game of rugby (Higgins et al., 2013). Variation in these findings compared to the results in this study may be attributed to the difference in muscle damage protocols used as this current study which used downhill running to induce muscle damage, whilst Higgins et al., (2013) used a simulated game of rugby.

5.2.5 Range of Motion

In the present study, there was no significant main effect for group for left knee range of motion. However, the main effects for time demonstrated that there was on average a 6% decrease in ROM of the left knee from 24 h post exercise to 72 h post exercise, however there was no significant decrease in ROM from Pre to 24 h post exercise. This shows that the muscle damaging protocol either had no effect on ROM of the left knee or there was a delay in the effects of muscle damage. This delay in the decrease in ROM may be attributed to the secondary increase in inflammation brought on by the disruption of Ca^{2+} homeostasis in the muscle. There was no significant interaction effect for left knee range of motion, which suggests that HWI and CWT had no effect on range of motion of the left knee compared to a CON group. Limited literature has investigated the effects of CWT and HWI on ROM. Similar to the findings of this study, French et al., (2008) found no significant differences in ROM of the knee following CWT compared to control. Decreases in ROM due to EIMD may be attributed to increases in limb circumference following muscle damaging exercise (Eston & Peters, 1999). No significant main effect for group and time was found for right range of motion and no significant interaction effect was found for group x time. It is unclear as to why no significant main effect for group and time was found to right range of motion only, however, it could be speculated this may be due to the participants in this study all being right leg dominant.

5.2.6 Limb Circumference

In the present study, no main effects for time or group were found for limb circumference of the rectus femoris, gastrocnemius and bicep femoris of the left and right leg. These findings suggest that the 40 min DHR had no effect on limb circumference. However, there was a significant interaction effect for right rectus femoris. In the CWT group limb circumference was 10% lower 48 h post DHR when compared to the HWI group. Similarly, previous studies have demonstrated a positive effect of CWT on limb circumference (Vaile et al., 2007, Vaile et al., 2008). Nevertheless, in support of findings in the present study HWI appears to have no effect on limb circumference post muscle damaging exercise (Vaile et al., 2008b, Vaile et al., 2007). This may be due to the mechanisms of HWI in which there is an increase in blood flow and essentially an increase in limb circumference. However, direct markers of muscle damage such as ROM and limb circumference may offer an insight to the indirect evidence of the mechanisms associated with EIMD. Increases in limb circumference and decreases in ROM could indicate the presence of inflammation and swelling (Proske and Morgan, 2001). Overall, the lack of literature demonstrating the link between the effects of HWI or CWT on ROM, limb circumference and skin temperature after a bout of eccentric muscle damaging exercise makes it difficult to determine whether an increase in limb circumference is linked to a decrease in range of motion, or whether there is a link between an increase in skin temperature and an increase limb circumference due to swelling and inflammation. In the present study, there is no link between the significant increases in left knee range of motion and the decrease in right rectus femoris limb circumference

5.2.7 Perceived Muscle Soreness

5.2.7.1 Pressure Pain Threshold and VAS

PPT may be used as an indirect marker of DOMS following muscle damaging exercise (Nosaka et al., 2002, Lavender & Nosaka, 2008). However, the most common indirect marker of perceived soreness is a VAS (Close et al., 2004). In the present study, there was a significant decrease in PPT in the left rectus femoris. There was a 19% decrease in PPT from Pre to 24 h post exercise, which indicates that the muscle damaging protocol was successful in producing muscle damage and subsequently, a sufficient increase in muscle soreness. At 72 h post exercise there was a 33% increase in PPT, which indicates a return to pre exercise values. However, there was no significant interaction effect, therefore indicating that the return to pre exercise values was not due to CWT or HWI.

There was a main effect for time for left bicep femoris, which found a 29% increase in PPT scores from 24 h to 72 h post exercise and a 31% increase from 48 h to 72 h post exercise. There was no significant decrease in PPT from Pre to 24 h indicating that the muscle damaging protocol did not affect PPT scores. However, a significant interaction effect was found at 72 h post muscle damaging exercise, as PPT in CWT group was 36% higher than the CON group and 35% higher than the HWI group. In the CON group PPT was 35% lower at 48 h post exercise than Pre. This indicates that PPT decreased post muscle damaging exercise and peaked at 48 h post exercise, without the intervention of water immersion. Alternatively, in the CWT group PPT at 72 h post exercise was 52% higher than Pre and 47% higher than Pre at 48 h. However, there was no significant decrease in PPT from Pre to 24 h in the CWT group. In fact, there was an initial increase in PPT mean scores from Pre to 24 h (7.3 KgF to 8.4

KgF), which may be attributed to the addition of CWT. For PPT of the right rectus femoris, PPT in the CWT group was 25% higher than the CON group, which indicates that CWT had a positive effect on PPT across all time points. For PPT of the right gastrocnemius, there was a significant main effect for time which demonstrated that at 72 h post exercise there was a 19% increase in PPT compared to Pre and 30% increase compared the 24 h and 16% increase compared to 48 h, however there was no significant interaction effect indicating that water immersion had no effect on PPT scores. It could be speculated that this increase in PPT may be due to participants becoming accustomed to the algometer used to assess PPT in this study. This could also be applied to PPT of the right bicep femoris with found that PPT increased by 29% from 24 h post exercise to 72 h post exercise.

VAS demonstrated a significant main effect for time. On average 24 h following the DHR, VAS was 55% higher compared to Pre DHR, which indicates an increase in perceived muscle soreness post muscle damaging exercise. Furthermore, there was an 86% increase in VAS from 24 h to 48 h and VAS scores were 225% at 24 h compared to 72h. There was no significant interaction effect, which suggests that contrast water therapy and hot water immersion had no effect on decreasing perceived muscle soreness post muscle damaging exercise. Similarly Vaile et al., (2007) reported a 37% increase in perceived muscle soreness in the CON and a 65% increase in the CWT group (Vaile et al., 2007) at 24 h post muscle damaging exercise. However in the same study, there was a 129% increase in perception of pain in the HWI group. This may be attributed to the significant difference in perceived pain values at Pre (p 0.03) between HWI and CWT (1.67 and 3.33 for HWI and CWT, respectively). As previously mentioned, although hydrostatic pressure does show to be beneficial for

the attenuation of EIMD, the temperature of the water may also affect perception of muscle soreness. For example, Montgomery et al (2008) demonstrated a significant decrease in perceived pain in the CWI group compared to the CON group. However, in contrast to the findings in the present study CWT was found to return participants to baseline values for perceived muscle soreness significantly more than HWI and CON (Montgomery et al., 2008). Differences in these findings compared to the present study could be due to the fact that Kuligowski and colleagues (1998) induced muscle damaging using eccentric elbow flexion (Kuligowski et al., 1998). Additionally, Vaile et al., (2008) also found a reduction in perceived muscle soreness at 24, 48 and 72 h post-exercise following CWT compared to CON. However, in agreement with the findings in the present study Vaile and colleagues found HWI to be ineffective in reducing the perception of pain following intense exercise. Although previous studies and the current study demonstrate beneficial effects of water immersion on perceived muscle soreness the aetiology of reduced perceived pain following water immersion remains to be elucidated.

5.5 Final Conclusions

Overall, it is apparent that the downhill run was successful in producing a sufficient amount of muscle damage as there are clear decrements in performance at 24 h post downhill run. The changes between groups for each marker are similar as the decrements in performance such as squat jump, flexibility, pressure pain threshold, perceived pain, and the increase in CK activity from Pre to 24 h followed by a gradual return to baseline values towards 72 h. These changes are also similar to the changes seen in the literature that demonstrates performance changes after exercise-induced muscle damage. As mentioned there is a plethora of literature that demonstrates the benefits of cold water immersion. However, the need to eliminate the extreme cold temperature for longer durations may be possible with contrast water therapy and hot water immersion. However, there is limited evidence that contrast water therapy and hot water immersion can attenuate performance decrements following a downhill run. However, in this study several markers improved suggesting that contrast water therapy and hot water immersion can improve recovery as flexibility and squat jump values peaked at 48 h in the control group compared to 24 h in the contrast water therapy and hot water immersion group as well as increase the rate of removal of creatine kinase from the blood.

5.5.1 Limitations

Typical to the majority of experimental research, the results of the studies in this thesis are moderately specific to the adherence of the participants involved and the experimental protocols employed. Therefore, the generalisation of the results found

and the implications of this study to the wider populations should be done with caution. Firstly, the number of participants within each group needs to be considered as a limitation. Another limitation is the absence of a cold water immersion group. Although it is clear that cold water immersion can help in alleviating the signs and symptoms of exercise-induced muscle damage, the addition of a cold water immersion group for this study would have been beneficial in increasing the number of participants as well as being able to compare the results of this study with the literature. Participants were provided with written pre-test procedures and asked to adhere to these for the duration of the study, and although compliance was checked at each visit, it is still possible that participants may not have adhered to these procedures. Furthermore, the participants used in the study were untrained, and therefore the findings cannot be extrapolated to a trained population. There was also a large variability of body mass of the participants, which in turn may have an effect on the extent of muscle damage produced during the downhill run, and subsequently may have an effect on the recovery or the rate of recovery. It is also essential to consider the low reproducibility of some of the indirect markers of muscle damage, and this could have obscured a true experimental effect in study 2.

5.5.2 Implications and Further Work

The practical implications of the findings of this study show that the indirect markers used in this thesis are ecologically valid, functional and measureable and can practitioners, coaches and athletes can replicate these tools in a field based setting. As well as allowing practitioners, coaches and trainers the chance to design a programme that can include recovery methods to attenuate muscle damage in order to assist an

athlete in their training or during competitions or tournaments. However, it can be said to only allow for the recovery interventions to be implemented during multi-day training and competitions and during the season to optimise recovery and performance. During off-season training and during non-competitions allow for the body to naturally heal from muscle damaging exercise promoting adaptation to exercise and hypertrophy and subsequently an increase in strength.

There are several potential areas for future research, which have emerged from this study, namely to investigate different duration, depths, and temperatures of water immersion protocols on both the signs and symptoms of exercise-induced muscle damage, and the effects on actual performance in a trained population. Based on the findings of this study, it is worth considering different recovery methods to help alleviate the signs and symptoms of exercise induced muscle damage, such as massage. Further research is required in order to determine whether massage may be superior to water immersion, using the same muscle damage protocol and markers of muscle damage. It is also important to test different populations (e.g. sedentary, recreation, athletes, and elite athletes) and well as different types of athletes (e.g. endurance vs. strength athletes) to determine the differences in muscle damage protocol as well as recovery interventions. Furthermore, future work should also investigate different markers of exercise-induced muscle damage, such as; other blood biochemical markers; myoglobin and interleukin-6, other performance measures such as; maximal voluntary contractions, sprint protocols, game simulation, time to exhaustion and a time trial.

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Appendices

Appendix 1: INFORMATION SHEET

The effects of contrast water therapy and hot water immersion on the signs and symptoms of exercise-induced muscle damage following a downhill run

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The purpose of the study is to determine the effects of hot water immersion (HWI) and contrast water therapy (CWT) on recovery after a single bout of downhill running (DHR).

What type of participant is needed?

We are looking for healthy individuals from the University of Bedfordshire to part-take in some form of exercise/training on a weekly basis. Must be over 18. Participants may NOT participate in this study if they have a history of coronary heart disease (or any other heart related problems), type 1 or type 2 diabetes, pregnancy, anaemia, eating disorders or current medication known to induce weight loss.

What will participants be asked to do?

You will first be required to do a $\dot{V}O_{2max}$ test to determine 70% of your treadmill velocity for the DHR. This will determine your speed for the duration of the DHR. You will also me required to perform test-measures. Between 4-7 days later you will be required to enter the labs again and perform the test-measures again (pre-test measures). These include range of motion of the knees using Goniometry, flexibility of the hamstrings using the sit and reach test and speed using a sprint protocol of 3 sprints over 10 metres. The test measures also include blood analysis via finger prick. This will measure your CK activity, blood glucose levels and blood lactate levels. Limb circumference and Pressure Pain Threshold (PPT) will be measured at 5 different points of both legs. You will then be required to perform 3 Maximal Isometric Forces using the KinCom. Once the test measures are complete you will perform a single bout of downhill running to induce muscle damage. You will run at -10% gradient for 40 minutes at 70% of your maximum treadmill velocity. Dependent on your group, you will be either placed in cold water, or hot water, alternate between both CWI and HWI (contrast water therapy) or used as a control group. 24hours later you will return to the labs for another bout of water immersion and post-test measures. 48hours after induction of muscle damage, you will return to the labs again for water immersion and post-test measures. Blood via finger prick will be taken before and

after the DHR and then at 24 hours after exercise and again at 48 hours and 72 hours after exercise. Blood will be taken in order to measure Creatine Kinase (CK) activity in the blood which increases during exercise and acts as an indicator of muscle damage.

What are the possible risks of taking part in the study?

The exposure to eccentric exercise (through downhill running) will cause severe muscle damage leading to pain and discomfort. You will be exposed to either extreme cold or/and extreme hot conditions which can lead to several severe climate related illnesses, such as hypothermia or heatstroke. You may not be accustomed to DHR therefore there will be an increase in injury. To minimise the risk of climate related illnesses, you will be required to wear a rectal thermometer so that the researchers can monitor your core temperature throughout the immersion.

What if you decide you want to withdraw from the project?

If you wish to withdraw from the study at any time, you may do so. No reason needs to be given and no questions will be asked.

What will happen to the data and information collected?

Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?

Questions are always welcome and you should feel free to ask myself, Brittany Erasmus or Dr. Lee Taylor any questions at anytime. See below for specific contact details.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

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Bedford Campus,
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Bedford

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Email: Brittany.erasmus@beds.ac.uk

Dr. Lee Taylor
Email: lee.taylor@beds.ac.uk

Appendix 2:

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

NAME:……(Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.
- I will be required to attend testing in the sport and exercise science laboratories on 5 separate occasions to complete the project.

As part of the study I will have to:

- Undergo familiarisation testing, which will include a $\dot{V}O_{2max}$ test, range of motion of the knees, flexibility and dynamic strength
- Perform an isometric voluntary contraction using the KinCom
- Perform a 45 minute downhill run which will induce muscle damage
- Have blood taken via finger prick on 5 different occasions
- Be immersed in either hot water, cold water or both hot and cold water
- Required to wear rectal thermometers on more than one occasion
- Have heart rate monitored throughout testing
- Give an indication of muscle soreness using a visual analogue scale of pain.

- I am aware of any risks that may be involved with the project.

- All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed:…… (Participant) Date:

Appendix 3:

PAR-Q

1. Have you ever been told by a doctor that you have a heart condition and advised only to participate in physical activity by your doctor?
2. Do you experience any chest pains when you participate in physical activity?
3. Have you recently experienced any chest pains whilst not participating in physical activity?
4. Do you ever lose consciousness?
5. Do you ever lose your balance as a result of dizziness?
6. Do you have any problems with you bones and joints that could cause further problems if you participate in physical activity?
7. Are you aware of any other reasons as to why you should not participate in physical activity?

Name: Signature: Date:

Appendix 4:

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?

b. Have you had jaundice within the previous year?

c. Have you ever had any form of hepatitis?

d. Have you any reason to think you may be HIV positive?

e. Have you ever been involved in intravenous drug use?

f. Are you a haemophiliac?

g. Is there any other reason you are aware of why taking blood might be hazardous to your health?

h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response in the box below:

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Name ……

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

The completed Medical Questionnaire (Par Q) and this Blood Sampling Form will be held in a locked filing cabinet in the Department of Sport and Exercise Science laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

Appendix 5:

PRE-TEST MEDICAL QUESTIONNAIRE

To be completed by all subjects before participating in practical sessions.

Name: …….

Age:…… Gender: M / F

1. Are you in good health? Yes / No
If no, please explain:

2.Are you pregnant or have you given birth in the last 6 months? Yes / No

3.How would you describe your present level of moderate activity?
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

4. Have you suffered from a serious illness or accident? Yes / No
If yes, please give particulars:

5.Are you recovering from an illness or operation? Yes / No
If yes, please give particulars:

6.Do you suffer, or have you ever suffered from:
Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No

Diabetes? Yes / No
Epilepsy? Yes / No
High blood pressure? Yes / No
Heart conditions or circulation problems:

(angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack,

other)?

Do you have chest pains at any time? Yes / No
Do you suffer from fainting/blackouts/dizziness? Yes / No
Is there any history of heart disease in your family? Yes / No

7. Are you currently taking medication ? Yes / No
If yes, please give particulars:

8. Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? If yes, please give particulars: Yes / No

9. Have you had to consult your doctor, or had hospital treatment within the last six months? Yes / No

10. Have you, or are you presently taking part in any other laboratory experiment? Yes / No

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment.
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and, if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

DECLARATION

I hereby volunteer to be a subject in experiments/investigations during the period of

_____.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, nor further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Name of subject (please print)
_____

Signature of Subject _____ Date:

Name of Experimenter (please print)_____

Signature of Experimenter _____ Date: